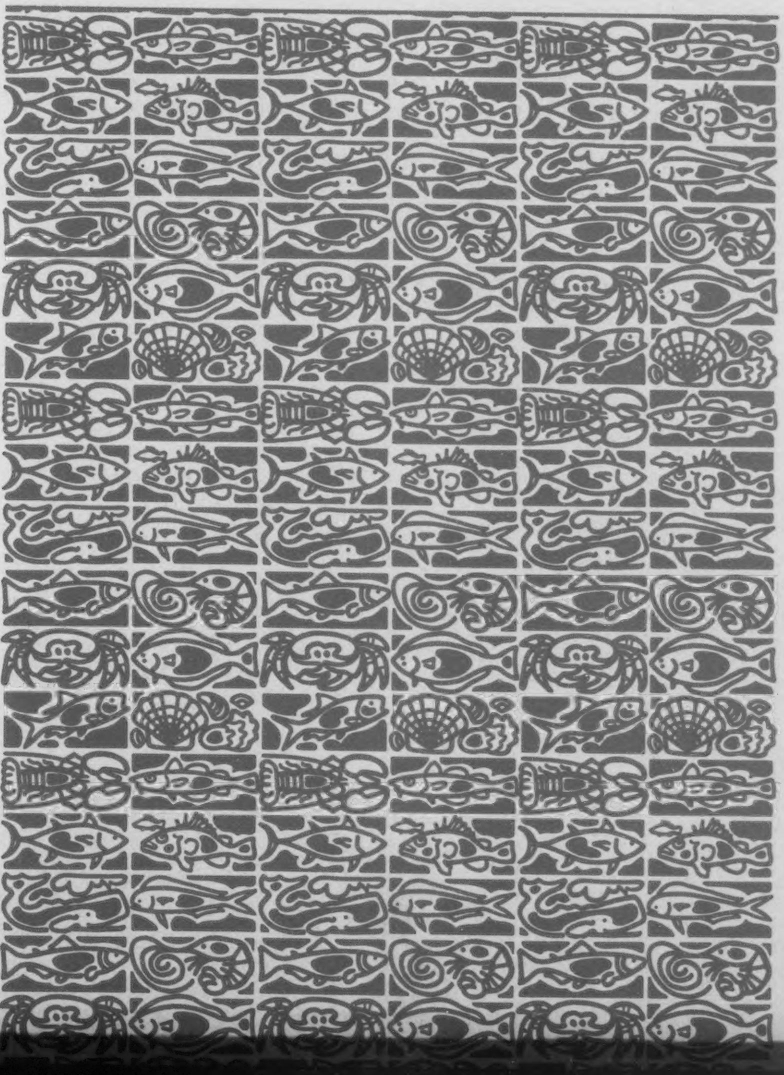
U.S. Department
of Commerce

Volume 101
Number 4
October 2003

Fishery
Bulletin



Fishery Bulletin

U.S. Department of Commerce
Donald L. Evans
Secretary

National Oceanic and Atmospheric Administration
Vice Admiral
Conrad C. Lautenbacher Jr., USN (ret.)
Under Secretary for Oceans and Atmosphere

National Marine Fisheries Service
William T. Hogarth
Assistant Administrator for Fisheries

The *Fishery Bulletin* (ISSN 0090-0656) is published quarterly by the Scientific Publications Office, National Marine Fisheries Service, NOAA, 7600 Sand Point Way NE, BIN C15700, Seattle, WA 98115-0070. Periodicals postage is paid at Seattle, WA, and at additional mailing offices. POSTMASTER: Send address changes for subscriptions to Fishery Bulletin, Superintendent of Documents, Attn.: Chief, Mail List Branch, Mail Stop SSOM, Washington, DC 20402-9373.

Although the contents of this publication have not been copyrighted and may be reprinted entirely, reference to source is appreciated.

The Secretary of Commerce has determined that the publication of this periodical is necessary according to law for the transaction of public business of this Department. Use of funds for printing of this periodical has been approved by the Director of the Office of Management and Budget.

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402. Subscription price per year: $55.00 domestic and $68.75 foreign. Cost per single issue: $28.00 domestic and $35.00 foreign. **See back for order form.**

Scientific Editor
Dr. Norman Bartoo

Associate Editor
Sarah Shoffler

National Marine Fisheries Service, NOAA
8604 La Jolla Shores Drive
La Jolla, California 92037

Managing Editor
Sharyn Matriotti
National Marine Fisheries Service
Scientific Publications Office
7600 Sand Point Way NE, BIN C15700
Seattle, Washington 98115-0070

Editorial Committee

Dr. Harlyn O. Halvorson	University of Massachusetts, Boston
Dr. Ronald W. Hardy	University of Idaho, Hagerman
Dr. Richard D. Methot	National Marine Fisheries Service
Dr. Theodore W. Pietsch	University of Washington, Seattle
Dr. Joseph E. Powers	National Marine Fisheries Service
Dr. Harald Rosenthal	Universität Kiel, Germany
Dr. Fredric M. Serchuk	National Marine Fisheries Service
Dr. George Watters	National Marine Fisheries Service

***Fishery Bulletin* web site: fishbull.noaa.gov**

The *Fishery Bulletin* carries original research reports and technical notes on investigations in fishery science, engineering, and economics. It began as the Bulletin of the United States Fish Commission in 1881; it became the Bulletin of the Bureau of Fisheries in 1904 and the *Fishery Bulletin* of the Fish and Wildlife Service in 1941. Separates were issued as documents through volume 46; the last document was No. 1103. Beginning with volume 47 in 1931 and continuing through volume 62 in 1963, each separate appeared as a numbered bulletin. A new system began in 1963 with volume 63 in which papers are bound together in a single issue of the bulletin. Beginning with volume 70, number 1, January 1972, the *Fishery Bulletin* became a periodical, issued quarterly. In this form, it is available by subscription from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402. It is also available free in limited numbers to libraries, research institutions, State and Federal agencies, and in exchange for other scientific publications.

U.S. Department
of Commerce
Seattle, Washington

Volume 101
Number 4
October 2003

Fishery Bulletin

Contents

Articles

721–731 **Baum, Julia K., Jessica J. Meeuwig, and Amanda C. J. Vincent**
Bycatch of lined seahorses (*Hippocampus erectus*) in a Gulf of Mexico shrimp trawl fishery

732–736 **Bjorndal, Karen A., Alan B. Bolten, and Helen R. Martins**
Estimates of survival probabilities for oceanic-stage loggerhead sea turtles (*Caretta caretta*) in the North Atlantic

737–744 **Chen, Yong, Margaret Hunter, Robert Vadas, and Brian Beal**
Developing a growth-transition matrix for the stock assessment of the green sea urchin (*Strongylocentrotus droebachiensis*) off Maine

745–757 **de Lestang, Simon, Norman G. Hall, and Ian C. Potter**
Reproductive biology of the blue swimmer crab (*Portunus pelagicus*, Decapoda: Portunidae) in five bodies of water on the west coast of Australia

758–768 **Dew, Jodi R., Jim Berkson, Eric M. Hallerman, and Standish K. Allen Jr.**
A model for assessing the likelihood of self-sustaining populations resulting from commercial production of triploid Suminoe oysters (*Crassostrea ariakensis*) in Chesapeake Bay

769–777 **Díaz-Jaimes, Pindaro, and Manuel Uribe-Alcocer**
Allozyme and RAPD variation in the eastern Pacific yellowfin tuna (*Thunnus albacares*)

778–789 **Govoni, John Jeffrey, Elisabeth H. Laban, and Jonathan A. Hare**
The early life history of swordfish (*Xiphias gladius*) in the western North Atlantic

790–799 **Heales, Donald S., David T. Brewer, You-Gan Wang, and Peter N. Jones**
Does the size of subsamples take from multispecies trawl catches affect estimates of catch composition and abundance?

The conclusions and opinions expressed in *Fishery Bulletin* are solely those of the authors and do not represent the official position of the National Marine Fisheries Service (NOAA) or any other agency or institution.

The National Marine Fisheries Service (NMFS) does not approve, recommend, or endorse any proprietary product or proprietary material mentioned in this publication. No reference shall be made to NMFS, or to this publication furnished by NMFS, in any advertising or sales promotion which would indicate or imply that NMFS approves, recommends, or endorses any proprietary product or proprietary material mentioned herein, or which has as its purpose an intent to cause directly or indirectly the advertised product to be used or purchased because of this NMFS publication.

800–808 **Laidig, Thomas E., Donald E. Pearson, and Lorraine L. Sinclair**
Age and growth of blue rockfish (*Sebastes mystinus*) from central and northern California

809–821 **Marin E., Baumar J., Antonio Quintero, Dany Bussière, and Julian J. Dodson**
Reproduction and recruitment of white mullet (*Mugil curema*) to a tropical lagoon (Margarita Island, Venezuela) as revealed by otolith microstructure

822–834 **McDonough, Christopher J., William A. Roumillat, and Charles A. Wenner**
Fecundity and spawning season of striped mullet (*Mugil cephalus* L.) in South Carolina estuaries

835–850 **Nelson, Peter A.**
Marine fish assemblages associated with fish aggregating devices (FADs): effects of fish removal, FAD size, fouling communities, and prior recruits

851–859 **Pajuelo, José G., José M. Lorenzo, and Muriel Gregoire**
Age and growth of the bastard grunt (*Pomadasys incisus*) Haemulidae) inhabiting the Canarian archipelago, Northwest Africa

860–873 **Punt, André E.**
Evaluating the efficacy of managing West Coast groundfish resources through simulations

874–888 **Purves, Martin G., David J. Agnew, Guillermo Moreno, Tim Daw, Cynthia Yau, and Graham Pilling**
Distribution, demography, and discard mortality of crabs caught as bycatch in an experimental pot fishery for toothfish (*Dissostichus eleginoides*) in the South Atlantic

889–899 **Stabenau, Erich K., and Kimberly R. N. Vietti**
The physiological effects of multiple forced submergences in loggerhead sea turtles (*Caretta caretta*)

900–909 **Stephenson, Peter C., and Norm G. Hall**
Quantitative determination of the timing of otolith ring formation from marginal increments in four marine teleost species from northwestern Australia

Notes

910–914 **Chan, Ricky W. K., Patricia I. Dixon, Julian G. Pepperell, and Dennis D. Reid**
Application of DNA-based techniques for the identification of whaler sharks (*Carcharhinus* spp.) caught in protective beach meshing and by recreational fisheries off the coast of New South Wales

915–922 **Ebert, Thomas A., and John R. Southon**
Red sea urchins (*Strongylocentrotus franciscanus*) can live over 100 years: confirmation with A-bomb ^{14}carbon

923–932 **Fulling, Gregory L., Keith D Mullin, and Carrie W. Hubard**
Abundance and distribution of cetaceans in outer continental shelf waters of the U.S. Gulf of Mexico

933–938 **Hata, David, and Jim Berkson**
Abundance of horseshoe crabs (*Limulus polyphemus*) in the Delaware Bay area

939–948 **Kerstetter, David W., Brian E. Luckhurst, Eric D. Prince, and John E. Graves**
Use of pop-up satellite archival tags to demonstrate survival of blue marlin (*Makaira nigricans*) released from pelagic longline gear

949–950 *2003 reviewers*

951–960 *2003 index*

961 *Subscription form*

Bycatch of lined seahorses (*Hippocampus erectus*) in a Gulf of Mexico shrimp trawl fishery

Julia K. Baum

Jessica J. Meeuwig

Amanda C. J. Vincent

Project Seahorse
Department of Biology
McGill University,
1205 Dr. Penfield Ave.
Montreal, Quebec, H3A 1B1, Canada
Present address (for J. K. Baum): Department of Biology
Dalhousie University
1355 Oxford St.
Halifax, Nova Scotia, B3H 4J1, Canada
E-mail address (for J. K. Baum): baum@mathstat.dal.ca

Abstract—Bycatch studies have largely ignored population level effects on fish species of little commercial interest. Here we analyze bycatch of the lined seahorse (*Hippocampus erectus*) in the bait-shrimp trawl fishery in Hernando Beach, Florida, providing the first fisheries data for this species. Based on catch per unit of effort (CPUE), size, sex, and reproductive status of trawled *H. erectus*, 1) approximately 72,000 seahorses were caught annually by this fleet, from a population of unknown size, 2) trawling affected population cohorts differentially because of temporal and spatial variation in CPUE and population size, and 3) a greater proportion of females than males was removed in trawling. Our findings suggest that trawling may affect seahorse populations through direct mortality, social disruption, and habitat damage. However, the lack of specific abundance or catchability estimates for *H. erectus* means that the precise impact of trawling on this fish remains uncertain. This paper focuses attention on the need for research and monitoring of small fishes that are caught incidentally in nonselective gear.

Manuscript approved for publication 2 June 2003 by Scientific Editor.

Manuscript received 26 June 2003 at NMFS Scientific Publications Office.

Fish. Bull. 101:721–731 (2003).

The incidental capture of marine organisms is now recognized as a serious problem in fisheries management and marine conservation (Alverson et al., 1994; IUCN, 1996; Alverson, 1997; Jennings and Kaiser, 1998; FAO, 1999). Shrimp trawl fisheries are the single greatest source of bycatch, accounting for 35% of the world's total bycatch (Alverson et al., 1994). Bycatch research has focused on marine megafauna, seabirds, and commercially important fish species (see as examples Polacheck, 1989; Graham, 1995; Weimerskirch et al., 1997; Julian and Beeson, 1998; Pikitch et al., 1998; Galloway and Cole, 1999; Diamond et al., 2000). The conservation impacts of bycatch for noncommercial fishes and invertebrate species remain largely unstudied (but see Chan and Liew, 1986; Pettovello, 1999; Milton, 2001). The few studies that have evaluated incidental capture of these species have focused on survival rates of individuals (Hill and Wassenberg, 1990, 2000; Kaiser and Spencer, 1995; Probert et al., 1997; Mensink et al., 2000) without addressing population level effects of bycatch. However, even species that comprise only a small portion of the bycatch in a fishery may experience significant impacts of incidental harvest on their population size and structure.

Seahorses are among those species inferred to be greatly affected by nonselective fishing gear, both because intense trawling often covers seahorse habitat and because their life history traits likely render these fishes vulnerable to overexploitation (Vincent, 1996). Most studied seahorse species are strictly monogamous (i.e. sexually and socially), meaning that removal can disrupt pairs and may reduce reproductive output (e.g. Vincent, 1995; Vincent and Sadler, 1995; Kvarnemo et al., 2000; Perante et al., 2002). Obligatory parental care by males, combined with relatively low fecundity, may reduce the potential for population recovery from overexploitation, although potentially high survival of young may also offset this apparent cost. In addition, sparse distributions and low mobility suggest that seahorses will be slow to recolonize depleted areas (Perante et al., 2002; Vincent et al.[1]).

Seahorses derived from bycatch appear to be contributing greatly to the large and growing international trade in these fishes (Vincent, 1996; Vincent and Perry[2]). Consumer demand for seahorses—both dried for traditional medicine and curiosity trades, and, less frequently, live for the aquarium trade—is very high (Vincent, 1996). Global demand for seahorses has surpassed supply and therefore trade has increased and expanded geographically

---

[1] Vincent, A. C. J., K. L. Evans, and A. D. Marsden. 2003. Home range behaviour of the monogamous Australian seahorse, *Hippocampus whitei*. Manuscript in review.

(Vincent, 1996; Vincent and Perry[2]), placing populations around the world under greater pressure. Much of this market demand is met from retention of incidental landings in shrimp trawls. Where no market has yet developed, incidentally caught seahorses are discarded and the survival rate for these discarded seahorses is unknown.

Trade records and anecdotal evidence from other countries indicate that the United States has imported and exported considerable numbers of both live and dried seahorses in recent years (Vincent, 1996; Vincent and Perry[2]). In Florida, the primary source of live seahorses in the United States (Larkin and Degner, 2001), these fishes ranked as the seventh most economically important ornamental fish group from 1990 to 1998, and seahorse landings rose 184% during this period, whereas landings of each of the more valuable fish groups declined (Adams et al., 2001). Many seahorses in the United States are probably obtained from bycatch of the shrimp trawl fisheries that operate in known seahorse habitats along the Atlantic coast of the United States.

Our objective was to document bycatch of the lined seahorse (*Hippocampus erectus*) in a live-bait shrimp trawl fishery. This fish is often retained for the aquarium trade. We quantify number, sex, size, and reproductive status of trawl-caught seahorses and examine how these parameters vary temporally and spatially. We also comment on potential conservation concerns resulting from this fishery.

Materials and methods

We focused our study on the lined seahorse (*Hippocampus erectus*) because it was caught much more often than its sympatric congenerics in the Gulf of Mexico—the longsnout seahorse (*H. reidi*) and the dwarf seahorse (*H. zosterae*). *Hippocampus erectus* is a large, deep-bodied seahorse (adult height 5.5 to 18.5 cm: Lourie et al., 1999) and has a geographic range that extends from southern Canada to Argentina. Most species of seahorse have short lifespans and low fecundity: *Hippocampus erectus* lives for about four years (Lourie et al., 1999) and has broods of about 100–1500 young (Teixeira and Musick, 2000). *Hippocampus erectus* is found in shallow waters and offshore to depths of over 70 m, primarily in mangroves and seagrass beds (Vari, 1982). Like many other seahorse species, *H. erectus* is listed as Vulnerable (A2cd) by the International Union for Conservation of Nature and Natural Resources (IUCN, 2002), based on suspected declines resulting from habitat degradation and exploitation. However, as is the case for many small fishes, there is little information known about the biology of *H. erectus*, and no fishery data exist for it.

Seahorse bycatch was assessed in the live-bait shrimp trawl fishery operating from Hernando Beach, Florida (Fig. 1). The fishery, using roller beam trawls, targets pink shrimp (*Penaeus duorarum*) at night in seagrass beds and relocates seasonally in Florida. Hernando Beach was chosen as our study site because it is a moderate-size fishing port with 31 licensed trawlers and is active during the summer sampling period. Boats were equipped with trawls that had a slotted roller along the bottom of the frame, and stainless steel finger bars attached vertically, 5 cm apart along the length of the frame, to limit collection of benthic substratum and other debris (Berkeley et al., 1985). Trawls were towed from each side of the vessel in four configurations: 1) one trawl per side, each measuring either 3.66 m, 4.27 m, or 4.88 m in length, or 2) two trawls per side, each measuring 3.66 m in length. Net mesh sizes were 3.18–3.81 cm and the tail bag stretched mesh size was 2.54–3.18 cm. Trawls usually lasted between 30 and 60 minutes, and fishermen made multiple successive trawls in a night. Shrimp were culled from the catch and held live onboard in aerated holding tanks. Most bycatch was discarded overboard, although some fishermen retained certain species, including seahorses, for sale as aquarium fishes.

Bycatch sampling and data set description

Data on seahorse bycatch in the live-bait shrimp fishery were collected on 95 fishing nights, from June to August 1998 and from June to July 1999, using three methods: 1) we sampled bycatch onboard on 50 nights; 2) we recorded seahorse bycatch data onshore on 14 nights from fishers who retained seahorses to sell; and 3) we received data from fishermen on their seahorse catches (including location, time, number of trawls, and number of seahorses per trawl) on a total of 31 nights. Onboard sampling was semistratified in that we targeted our sampling to cover all lunar and tidal phases and a variety of areas. However, we were dependent on fishersmen's decisions about the time and location of tows. We also collected anecdotal information from 14 experienced fishermen about seahorse catches over time.

During onboard sampling, we recorded the number of seahorses caught, start and end time of trawl, depth and location (Loran co-ordinates) of trawl, tidal and lunar phases, and presence or absence of all bycatch species, including biogenic habitat species. *Hippocampus erectus* found in the catch were placed in a container of surface water while measurements were made. They were then released, except when fishermen chose to retain them for sale. We measured, weighed, determined the sex, and recorded the life history stage, reproductive status, and any injury for each seahorse. Measurements were taken according to Lourie et al. (1999) and included standard length, defined as the length from the tip of the snout to the opercular ridge and from the opercular ridge to straightened tail tip. Seahorses that had lost tail rings were not included in the length analysis. Unless precluded by logistic constraints, wet weights were obtained onboard by using a 60-g Pesola spring scale and onshore by using a 200-g Ohaus electronic balance. Adult males were distinguished from females and juveniles by the presence of a brood pouch. The standard length of the smallest seahorse with a brood pouch (105.3 mm) was used as the division between adults and juveniles with the assumption that males and females matured at the same size. Such an assumption may overestimate the

---

[2] Vincent, A. C. J., and A. Perry (eds.). 2003. Global trade in seahorses. Manuscript in prep.

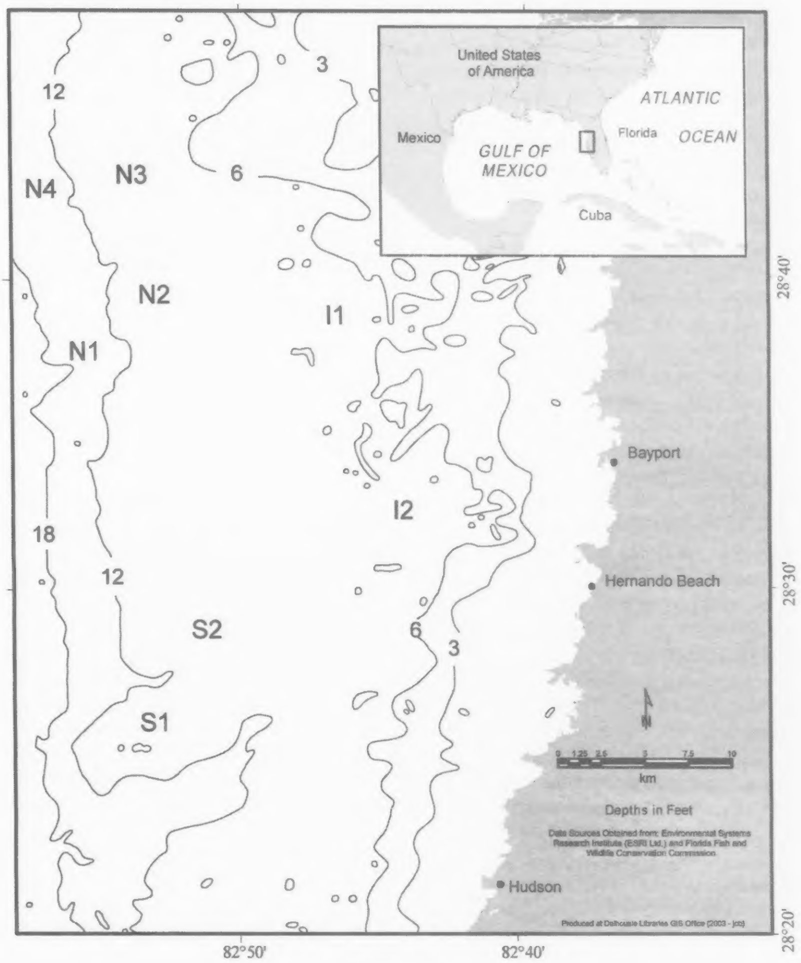

12
3
6
N4
N3
N2
N1
I1
I2
18
12
S2
6
3
S1
United States of America
ATLANTIC
OCEAN
Mexico
GULF OF MEXICO
Florida
Cuba
Bayport
Hernando Beach
Hudson
0 1.25 2.5 5 7.5 10
km
Depths in Feet
Data Sources Obtained from: Environmental Systems Research Institute (ESRI Ltd.) and Florida Fish and Wildlife Conservation Commission.
Produced at Dalhousie Libraries GIS Office (2003 - jcb)
82°50'
82°40'
28°40'
28°30'
28°20'

Figure 1

Map of the eastern Gulf of Mexico, showing the study site of Hernando Beach on the western coast of Florida. The shore is shaded; additional solid lines indicate depth contours in feet. Letter and number combinations (e.g. N3) represent fishing areas along the coast.

number of adult females because males that matured after that size would have been included in our analysis as females. We defined males as reproductively active if they were pregnant or had recently released young (as indicated by a loose pouch). Female reproductive state was not included in this analysis because it is difficult to determine reliably. We defined mortality to include seahorses already dead when the net was hauled and those that died onboard.

We evaluated temporal and spatial patterns in catch per unit of effort (CPUE), standard length, population structure, and reproductive status of the seahorse bycatch. Specifically, we tested 1) temporal effects of year, lunar and tidal phase, and 2) spatial effects of area and depth. In our estimates of seahorse CPUE, we used standard length instead of biomass because female weight changes with egg hydration and male weight increases greatly when carrying embryos. Calculating CPUE per length (m) of roller beam trawl controlled for variation in gear size. The sampling unit was thus defined as the total or cumulative standard length of seahorses caught during each tow (per hour), per meter of trawl (per meter). Lunar phases were defined as continuous variables by converting lunar day to its angle, θ, based on a cycle of 29.5 days (=360°), with new moon defined as $\theta = 0°$. These angles were then converted to their cosine and sine functions for inclusion in linear regression (deBruyn and Meeuwig, 2001). Tides were semidiurnal in the Gulf of Mexico, ranging 1.3 m in tidal level in the study area. High and low tides were defined as each lasting two hours, with the remaining time classified as ebb or flood accordingly. Spatial effects were analyzed by dividing the total fishing ground into eight subareas according to their position with respect to the depth contour and by identifying discrete geographical clusters of trawls (Fig. 1). We then compared variation among fishing areas within years for those areas with at least five observations (areas I1, I2, N1, N2, S1, S2 in 1998, and areas N1, N3 and N4 in 1999). Interannual spatial comparisons were not possible because there was little overlap in sampled areas between the two years.

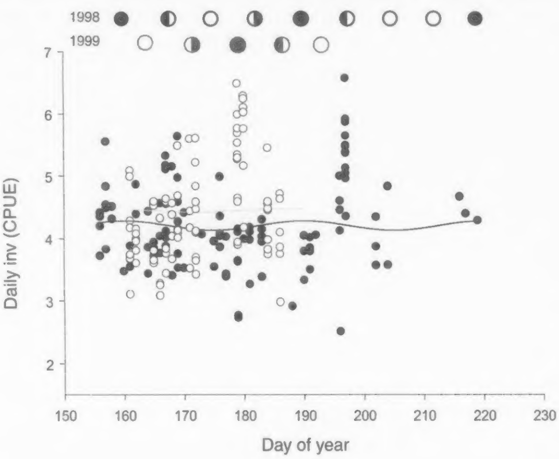


Figure 2

Variation in mean CPUE estimates by lunar phase transformed to inverse hyperbolic sine. The curve represents the predicted values as a function of the cosine (1998) and sine (1999) of day. The lunar phases for both years are indicated. Filled circles and black line are for 1998; open circles and gray line are for 1999.

Statistical analysis

We based CPUE estimates on all trawls (n=445). Statistical analyses evaluating temporal and spatial variation in seahorse bycatch included only those trawls with nonzero seahorse observations (n=205). The analysis required the data or their residuals to be normal; this was achieved by inverse hyperbolic sine transformations following exclusion of zeros (Zar, 1996). It should be noted that by excluding zeros we overestimated seahorse CPUE and we lost information about areas where seahorses were absent or rare. Our analyses should be interpreted as applying to locations where and times when seahorses were found in sufficient numbers as to be caught.

We examined the data using ANOVA, ANCOVA, linear regression and chi-square analyses (Zar, 1996; SYSTAT, version 7.0, SPSS Inc., Chicago, IL). All two-way and three-way ANOVAs included tests for interactions. We used a general linear model because the data were unbalanced. Interactions were removed from the model if they were found to be nonsignificant. Models were then rerun, followed by pairwise Tukey tests to indicate where significant differences occurred (Zar, 1996; SYSTAT, version 7). We report results for the final ANOVA only. The Yates correction was applied to 2×2 chi-squares (Zar, 1996). All significance levels were set to reject H_0 at $P<0.05$ and all means are reported with standard errors.

Results

Bait shrimp fishermen trawled from 11 to 24 km offshore, between 1.8 and 6.4 m deep water (mean=3.76 ±0.87 m). Fishermen typically left port between 17:00 and 19:00 and spent 5.8 ±0.23 h actively trawling per night (n=50 nights). Trawls lasted 40.2 ±11.4 min (n=445) on average, and fishermen usually set 8 to 9 trawls per night. Distance trawled could not be estimated because we were unable to track trawler trajectories continuously and because they changed direction during the tows. The benthic habitat was composed primarily of seagrass (*Thalassia testudinum*) but also included algae, coral, and sponge. Bycatch included at least 118 species of fishes, invertebrates, and marine flora.

Catch per unit of effort

Hippocampus erectus was the only seahorse species commonly caught in this fishery. Almost half of the trawls (46%) caught *H. erectus*, and the number per trawl ranged from 0 to 16, whereas no *H. reidi* and only two of the much smaller species, *H. zosterae*, were caught. In total, 916 *H. erectus* were caught during the 95 documented fishing boat nights of the two fishing seasons, resulting in an overall mean of 9.64 seahorses per fishing boat night. Mean CPUE for *H. erectus* was 24.25 ±2.15 mm/(h×m), about one and a half seahorses per hour per boat. If only trawls with seahorses were included, CPUE was 52.52 ±3.80 mm/(h×m), or about three seahorses per hour per boat (n=205). Very high CPUE was recorded on three nights: 16 July 1998 (mean CPUE=122.0 ±22.5 mm/(h×m), n=12 trawls), 28 June 1999 (mean CPUE=118.1 ±24. mm/(h×m), n=12 trawls) and 30 June 1999 (mean CPUE=154.9 ±36.6 mm/(h×m), n=8 trawls). Bycatch is characterized by a high number of low catches and infrequent large catches. Because the large catches more likely reflect the spatial-temporal distribution characteristics of fish stocks rather than outliers of the data (Ortiz et al. 2000), we analyzed the entire dataset and then tested the robustness of our models by excluding these three nights in order to assess their influence on the CPUE patterns.

CPUE of nonzero trawls varied between years and with lunar phase (Table 1), but not with tidal phase (P=0.15). Trawls captured significantly more seahorses in 1999 than in 1998 ($P<0.0005$). The effect of lunar phase varied between years: CPUE was highest on the lunar third quarter in 1998, but only weakly significant and had slightly higher CPUE on the full moon in 1999 (Fig. 2). The temporal variation in CPUE was largely driven by the three high CPUE nights, but the effect persisted when these were excluded (Table 1).

Table 1

General linear model of effects of year, lunar phase, and area on the CPUE (mm/(h×m) for nonzero *H. erectus* bycatch trawls in the Hernando Beach bait-shrimp trawl fishery.

	CPUE by trawl			CPUE by trawl (excluding CPUE of three highest nights)		
Source	*n*	*F*	*P*	*n*	*F*	*P*
Year	205	15.2	<0.0005	173	3.9	0.049
Lunar phase	205			173		
cos(θ)		19.1	<0.0005		4.1	0.045
sin(θ)		8.9	0.003			
1998 areas (I1, I2, N1, N2, S1, S2)	116	7.4	<0.0005	105	2.3	0.037
1999 areas (N1, N2, N3, N4)	87	8.3	<0.0005	68	0.3	0.85

In both years, there were significant differences in nonzero CPUE trawls among sites. In 1998, CPUE was significantly higher in S2 than in I2, N1, N2 and S1 (Fig. 3A, Table 1). In 1999, CPUE was significantly higher in area N1 than in areas N3 and N4 (Fig. 3B, Table 1). However, both of these spatial patterns, like the lunar patterns, were driven primarily by the high CPUE nights. Removing the three outliers left a significant difference in CPUE by area in 1998 only (Table 1). CPUE did not vary with depth of the fishing ground (P=0.67).

Size of seahorses

Mean standard length of adults (standard length ≥105.3 mm) was 139.5 ±21.7 mm (n=465, range 105.3–202 mm) and mean weight was 11.6 ±5.5 g (n=232, range 3–31 g). *Hippocampus erectus* was sexually dimorphic: males had a brood pouch and were significantly longer than females (n=465, P<0.0005, Table 2), and had a greater weight to standard length ratio, although this latter difference was relatively weak (P=0.04). Juveniles (standard length <105.3 mm) had a mean standard length of 83.3 ±16.7 mm (n=65, range 41.4–105 mm) and a mean weight of 2.4 ±1.0 g (n=38, range 0.9–4.0 g).

In adults, standard length varied by year (n=425, F=7.1, P=0.008) and by lunar phase (n=425, cos(θ):F=5.4, P=0.02, sin(θ):F=7.7, P=0.006). Mean standard length was greater in 1998 than in 1999 and highest on the new moon. In 1998 significantly larger adult seahorses were caught in areas I2 and S2 than in I1 and S1 (n=229, F=13.7, P<0.0005; Fig. 4). There was no effect of area in 1999 (n=212, F=1.9, P=0.14). Standard length was not related to depth or tide.

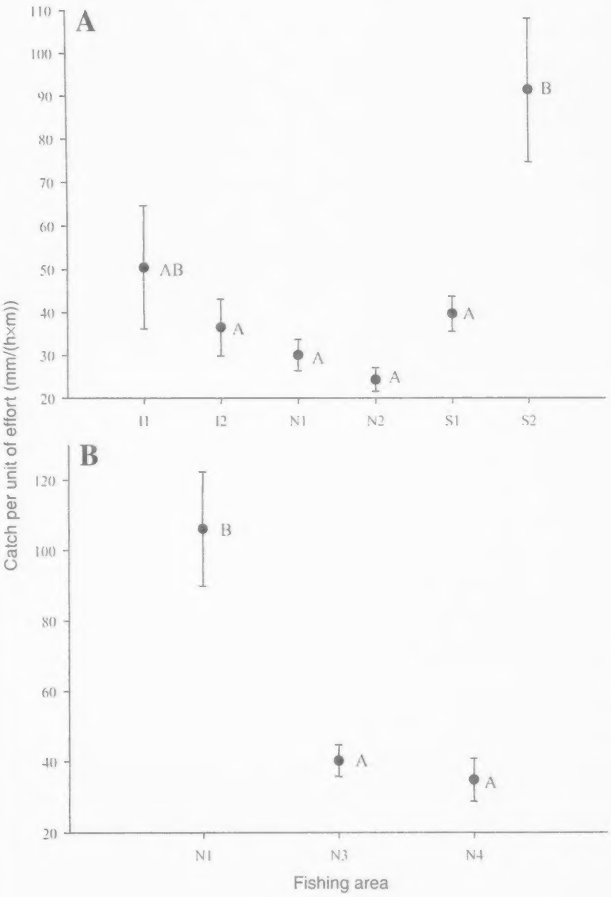


Figure 3

Catch per unit of effort (±standard error) as a function of fishing area in (**A**) 1998 and (**B**) 1999. Letters indicate where significant differences lie at P<0.05.

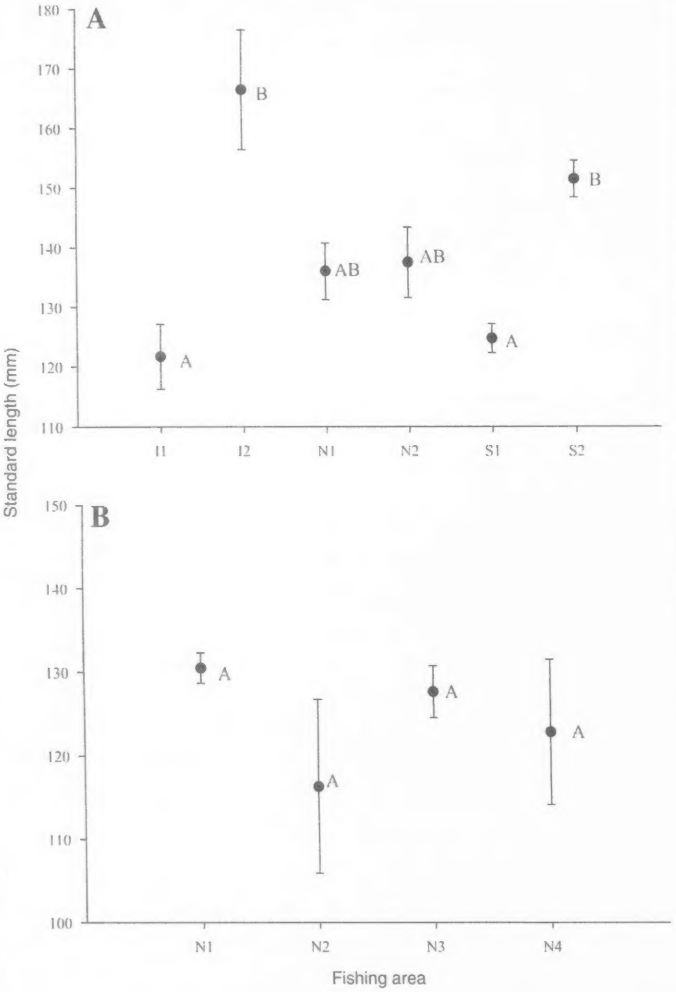


Figure 4

Standard length ±standard error as a function of fishing area in (**A**) 1998 and (**B**)1999. Letters indicate where significant differences lie at $P<0.05$.

Population structure

We assessed population structure in terms of size cohorts, life history stages, sex ratio (males/total) and reproductive status. Standard-length frequency histograms suggested that the 1998 bycatch was composed of three year classes but the largest of these was not evident in 1999 (Fig. 5). Of 530 seahorses measured, 87.7% were considered to be adults. The ratio of juveniles to adults did not differ by year ($P>0.10$) or tidal phase ($P>0.90$). However, significantly more juveniles were caught during the first quarter than during other lunar phases (Table 3). Fishing area had a significant effect on size class, with the highest numbers of juveniles caught in N2 and N3 (Table 3).

The sex ratio (males as fraction of total) of 0.42 differed significantly from a 1:1 ratio ($\chi_c^2=19.56$, df=1, $P<0.001$). The sex ratio did not vary temporally or spatially (Table 3), but it did vary as a function of size class. There were proportionally more males in the larger size class (≥150 mm, 0.61) than in the smaller size class (<150 mm, 0.30) ($\chi_c^2=14.95$, df=1, $P<0.005$).

About 25% of the male seahorses captured in 1998 were considered to be reproductively active, whereas fewer than 1% were reproductively active in 1999. Indeed, male reproductive activity was higher in 1998 than 1999 even after controlling for smaller male size in 1999 (Table 3). The proportion of reproductively active males did not vary with lunar or tidal phase but did vary significantly with area

Table 2

Descriptive statistics (sample size [*n*], mean, standard deviation [SD], minimum [min] and maximum [max]) for standard length and weight of female (F) and male (M) seahorses. *P* values and sample sizes (*n*) indicate results of *t*-tests evaluating sexual dimorphism in SL and weight.

	Sex	*n*	mean	SD	min	max
Standard length (mm)	M	201	146.1	21.9	105.3	200.5
$P<0.0005$, $n=465$	F	264	134.4	20.1	105.3	202.0
Weight (g)	M	105	12.5	5.0	6.0	27.0
$P<0.002$, $n=232$	F	140	11.0	5.8	3.0	31.0

Table 3

Contingency tables on effects of year, lunar phase, and area on population structure of seahorses in the bycatch of the Hernando Beach bait shrimp trawl fishery. Yates corrections were applied to 2×2 contingency tables.

	Juveniles:Adults			Sex ratio			Reproductive state		
Source	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	P
Year	1	2.46	>0.10	1	0.15	>0.50	1	27.89	<0.001
Lunar phase	3	11.00	<0.025	3	3.69	>0.25	3	7.80	>0.05
Area	3	8.84	<0.05	7	13.3	>0.05	2	21.64	<0.001

(Table 3); almost half of the reproductively active males were found in one area (S2), and 83% of them were caught on one of the three nights with very high CPUE (16 July 1998).

Mortality and injury

Fewer than 1% of seahorses died during tows or sorting, but 4.7% ($n=28$ of 588 seahorses) lost tail rings. The mean loss was 22 of the usual 36 tail rings (Lourie et al., 1999), or 61% of the tail (range=8–31 rings lost). Approximately 14% of the losses ($n=4$ of 588 seahorses) appeared to be the result of new wounds, probably caused by the focal trawl. There was no effect of year ($P=0.25$), sex ($P=0.75$), or reproductive status ($P=0.75$) on incidence of seahorse injury. Postrelease mortality is unknown.

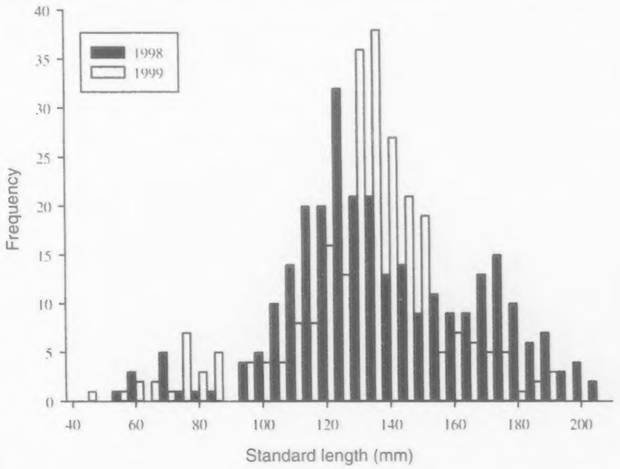


Figure 5

Length-frequency histogram for seahorse bycatch in 1998 and 1999.

Discussion

Direct effects of the trawl fishery on seahorse mortality

We estimate that this fleet catches almost 72,000 seahorses incidentally per annum, based on the mean CPUE and given that 31 boats fished approximately 240 nights per year. Most seahorses were returned to the wild in the Hernando Beach fishery, but this may not be the case in other live-bait shrimp trawl fleets in Florida (Vincent, pers. obs.). We could not determine the potential fishing-induced mortality for the Hernandez Beach *H. erectus* population, even when all trawled seahorses were retained, because seahorse catchability in roller beam trawl gear is unknown and no studies have estimated *H. erectus* density in the wild. Focal behavioral studies on congenerics similar in size to *H. erectus* have found varied densities: *H. comes*, an exploited species associated with coral reefs in the tropics, had localized densities of 0.019/m^2 in a marine protected area, and much less elsewhere (Perante et al., 2002), and an unexploited species, *H. whitei*, had localized densities of 0.088–0.215/m^2 in a study area, and no seahorses were found over large adjacent areas (Vincent et al.[1]). Our study also suggests very patchy distributions of *H. erectus* (54% of the trawls had no seahorses at all and the number of seahorses per trawl set ranged from 0 to 16).

Although variation in CPUE may reflect differential catchability by habitat, we suggest that in areas where seahorses were caught, temporal rather than spatial effects drove CPUE. It is difficult to make conclusions about variation in CPUE because data were unbalanced, in that the areas trawled differed between years and among lunar phases. However, analysis of variance on a subset of data for three sites (N1, N2, N3) on three lunar phases (1st quarter, full moon, 3rd quarter) for which we had data in both years (n=149 trawls), indicated that there was a strong effect of year, a weaker effect of lunar phase, and no effect of site. These results suggest that CPUE was mainly affected by temporal variation. Lunar patterns in CPUE as a result of fish behavior and ecology are common (e.g. Parrish, 1999). This would be consistent with observations for other species of seahorses: *H. comes* in the Philippines (Vincent et al.[3]) and *H. spinosissimus* and *H. trimaculatus* in Vietnam (Meeuwig et al.[4]) exhibited patterns in CPUE with respect to lunar phase, although these species were also distributed in patches in space.

Data from this study suggest that the *H. erectus* population was spatially structured. In 1999, the mean size of incidentally caught adult seahorses decreased, reflecting the absence of the largest size class of males and an increase in smaller females that year (Fig. 5). We attribute this difference to spatial structuring: the shallower areas (I2, S2) where the largest male and female seahorses were caught in 1998 were not fished during the 1999 sampling season. Most of the seahorse bycatch were adult *H. erectus;* the dearth of juvenile *H. erectus* (and dwarf seahorses, *H. zosterae*) in the trawls reflects low catchability or retention due to mesh size. Similar proportions of juvenile seahorses were caught over the two sampling seasons. The ratio of juveniles to adults appears to be temporally influenced (proportionally more juveniles were caught on new moons), but this variation probably also reflects spatial structuring because these trawls occurred primarily in deeper offshore areas (N2, N3) that were fished almost exclusively during this lunar phase. Perhaps *H. erectus* undergoes ontogenetic movement, between juvenile and adult life history stages, and adults maintain site fidelity. Spatial size structuring probably also occurs in other seahorse species, for the entire population and for adults alone (*H. comes*, Meeuwig[5]; *H. guttulatus,* Curtis[6]). A better understanding of the spatial structuring of populations could allow for spatial control of fishing effort to minimize bycatch.

We found a consistent, female-biased sex ratio in the catch across the two years of our study, with only 42% males. This bias may reflect the sex ratio of the *H. erectus* population: a similar sex ratio (40% males) was found in a population of *H. erectus* in Chesapeake Bay, Virginia (Teixeira and Musick, 2000). Female-biased sex ratios have also been found in *H. zosterae* (33% males) when sampled by pushnet (Strawn, 1958), and in *H. abdominalis* studied underwater in Australia (Martin-Smith[7]). Many other wild populations of seahorses studied underwater, however, have documented equal numbers of males and females (*H. breviceps*: *H. comes*: Moreau and Vincent[8]; Perante et al., 1998; *H. reidi*: Dauwe, 1993; *H. whitei*: Vincent and Sadler, 1995). Sexual dimorphism in *H. erectus* was too slight to explain different catchability of the two sexes and would, in any case, have favored the capture of males. The disproportionate catch of females could have arisen from spatial segregation by sex; the greater catches of reproductively active males in shallower areas suggests that males may spend most of their time inshore of the trawled area. We also cannot discount the possibility that some seahorses classified as females may have been immature males, and the sex ratio in the population could in fact be 1:1.

The proportion of reproductively active seahorses in the bycatch was lower than expected, particularly in 1999. Our study occurred during summer, within the breeding season for the congeneric and sympatric *H. zosterae* in Florida (February to October; Strawn, 1958), and for *H. erectus* in Chesapeake Bay (May to October; Teixeira and Musick, 2000; Vincent, personal obs.). Males of all studied seahorse species were reproductively active almost continuously throughout the breeding season (Dauwe, 1993; Nijhoff, 1993; Vincent and Sadler, 1995; Perante et al., 2002), often remating the same day that they release their young (Vincent and Sadler, 1995). In our study, trawling may have occurred outside the primary breeding areas for male *H. erectus,* but catches of reproductively nonactive adult males during the breeding season also suggest that repeated trawling may have disrupted breeding in the population. A further indication of possible spatial structuring in the population (by reproductive status and size) is that almost half of the reproductively active males caught in 1998 were found in S2, the shallowest area; this area was not sampled in 1999 when few reproductively active males were found. Such spatial structuring offers the possibility of trawling outside the breeding area.

---

[3] Vincent, A. C. J., J. J. Meeuwig, M. G. Pajaro, and N. C. Perante. Seahorse catches in the central Philippines: characteristics and conservation implications. Manuscript in prep.

[4] Meeuwig, J. J., D. H. Hoang, T. S. Ky, S.D. Job, and A. C. J. Vincent. Bycatch landings of seahorses in central Vietnam. Manuscript in prep.

[5] Meeuwig, J. J. Life history parameters of the exploited seahorse *Hippocampus comes*: a length based analysis. Manuscript in prep.

[6] Curtis, J. 2002. Unpubl. data. Project Seahorse, Fisheries Center, The University of British Columbia, 2204 Main Mall, Vancouver, BC, V6T 1Z4, Canada.

[7] Martin-Smith, K. 2002. Unpubl. data. Project Seahorse, Fisheries Center, The University of British Columbia, 2204 Main Mall, Vancouver, BC, V6T 1Z4, Canada.

[8] Moreau, M-A., and A. C. J. Vincent. 2000. Unpubl. data. Project Seahorse, Fisheries Center, The University of British Columbia, 2204 Main Mall, Vancouver, BC, V6T 1Z4, Canada.

Indirect effects of the trawl fishery on mortality

Direct immediate mortality from trawling and culling was rather low, probably in part because trawl sets were of very short duration in this live-bait fishery. More importantly, most *H. erectus* caught in the Hernando Beach fishery were returned to sea, rather than retained as in some other Florida trawl fisheries (Vincent, pers. obs.). Indirect impacts of the fishery may, however, be considerable.

Seahorses caught in trawls may experience high postrelease mortality. A study in the live-bait shrimp trawl fishery in Tampa Bay, Florida (Meyer et al., 1999), found that only one of four of the congeneric dwarf seahorse (*H. zosterae*) caught as bycatch were alive in the holding tank of seawater 36 hours after collection (Meyer[9]). Such tows lasted only 5 minutes (Meyer et al., 1999); therefore trawl-induced mortality could be greater in the Hernando Beach fishery (with trawls of 30–50 minutes), although *H. erectus* are larger and perhaps more robust than *H. zosterae*. Like other discarded bycatch, seahorses may also be subject to intense predation upon release. Predation on discarded fish has been observed on prawn trawlers in Australia (Hill and Wassenberg, 1990) and within the bait shrimp fishery of Tampa Bay (Meyer et al., 1999). Captains of bait-shrimp boats concurred that this is commonplace in the Hernando Beach fishery, and we frequently observed bottlenosed dolphins (*Tursiops truncatus*) and schools of ladyfish (*Elops saurus)* swimming alongside the boats, feeding on discarded bycatch.

Trawling may significantly disrupt seahorse populations, particularly if they are spatially structured, as the present study suggests. The disproportionate removal of females could reduce mating opportunities, especially if *H. erectus* is monogamous, as are most studied seahorse species (e.g. Vincent, 1995; Vincent and Sadler, 1995; Kvarnemo et al., 2000; Perante et al., 2002). Trawling, on account of repeated intrusion onto breeding grounds, could also disrupt courtship and negatively affect reproduction, In heavily exploited areas of the fishery where fishermen repeatedly trawl productive areas, seahorses may face cumulative stress. For example, tail injuries are likely a serious wound for seahorses, given that their tails are essential to grasp holdfasts and may play a key role in mating competition, as they do with *Hippocampus fuscus* (Vincent, 1994).

Benthic habitat degradation is another potential indirect effect of live-bait shrimp trawling on seahorses. Bottom-fishing gear can reduce habitat complexity by removing emergent epifauna, smoothing sedimentary bedforms and by removing structure-forming species such as corals and sponges (Hutchings, 1990; Auster et al., 1996; Auster and Langton, 1999; Thrush and Dayton, 2002). Roller beam trawls also affect habitat complexity by redistributing macroalgae and seagrass (Meyer et al., 1999). We estimated that seagrasses comprised between 50% and 80% of the volume of the catch for each trawling operation. Although roller beam trawls are assumed to have low impact on seagrass habitat (Tabb and Kenny, 1969; Meyer et al., 1999), the effects of long-term repetitive trawling have not been tested, and it is possible that species composition and abundance, including that of *H. erectus*, have been adversely affected (Watling and Norse, 1998).

Summary

Despite the relatively low direct mortality of seahorse per boat, the live-bait trawl fishery has the potential to affect seahorse populations, both directly and indirectly. The key question is whether the level of exploitation, and subsequent impacts, represents a conservation concern. Our evidence is inconclusive. Perhaps only the skewed sex ratio and low proportion of reproductively active males suggest a potential problem. However, fishermen have consistently reported that seahorse catch per boat has declined greatly over the past two decades in this area. Effects of trawling are also almost certainly greater in food shrimp trawl fisheries, which trawl with much larger gear for longer periods, and obtain substantially more bycatch, with higher mortality. Our analysis should thus be seen as a first step in identifying areas for which more information is needed, specifically estimating abundance and fishing mortality, and understanding spatial structuring in *H. erectus*.

This paper focuses attention on the need for research on and monitoring of small fishes that may be affected by nonselective fishing gear. Management responses to minimize bycatch have focused primarily on seabirds, sea turtles, and commercially important finfishes, but trawl fisheries may also have significant impacts on the many small marine organisms obtained as bycatch, even if they comprise only a small proportion of the bycatch. Bycatch excluder devices are unlikely to be effective in reducing incidental catches of these species. Temporal variation in CPUE and spatial population structuring, as observed in our present study for *H. erectus,* suggest that time-area closures may be a pragmatic solution for reducing incidental catch.

Acknowledgments

This paper is a contribution from Project Seahorse. We thank Jana Schulz for her assistance with fieldwork, Daniel and Patricia Mohr for their support during fieldwork, James Boxall for preparing the map, A. DeBruyn, L. Crowder, M. Kaiser, and an anonymous reviewer for providing helpful comments on an earlier draft of this manuscript. This study would not have been possible without the cooperation and support of many of the shrimp boat captains and crew in Hernando Beach. This research was funded through an NSERC summer undergraduate award to JKB, support from the Community Fund (UK) and Guylian Chocolates Belgium for JJM, and an NSERC operating grant to ACJV.

Literature cited

Adams, C., S. Larkin, and D. Lee.
2001. Volume and value of marine ornamentals collected in Florida, 1990–98. Aquar. Sci. Conserv. 3(1–3):25–36.

---

[9] Meyer, D. 1999. Personal commun. NOAA Center for Coastal Fisheries and Habitat Research, Beaufort Laboratory, 101 Pivers Island Road, Beaufort, NC 28516.

Alverson, D. L.
1997. Global assessment of fisheries bycatch and discards: a summary overview. *In* Global trends: fisheries management (E. K. Pikitch, D. D. Huppert, and M. P. Sissenwine, eds.), 115–125 p. Am. Fish. Soc. Symp., Vol. 20.

Alverson, D. L., M. H. Freeberg, S. A. Murawski, and J. G. Pope.
1994. A global assessment of fisheries bycatch and discards, 233 p. FAO Fish. Biol. Tech. Pap. 339. FAO, Rome.

Auster, P. J., and R. W. Langton.
1999. The effects of fishing on fish habitat. *In* Fish habitat: essential fish habitat (EFH) and rehabilitation (L. Benaka, ed.), p. 150–187. Am. Fish. Soc. Rep. 22.

Auster, P. J., R. J. Malatesta, R. W. Langton, L. Watling, P. C. Valentine, C. L. S. Donaldson, E. W. Langton, A. N. Shepard, and I. G. Babb.
1996. The impacts of mobile fishing gear on seafloor habitats in the Gulf of Maine (Northwest Atlantic): implications for conservation of fish populations. Rev. Fish. Sci. 4:185–202.

Berkeley, S. A., D. W. Pybas, and W. L. Campos.
1985. Bait shrimp fishery of Biscayne Bay, 16 p. Fla. Sea Grant Ext. Prog. Tech. Pap. 40. Florida Sea Grant, Gainesville, FL.

Chan, E. H., and H. C. Liew.
1986. Characteristics of an exploited tropical shallow-water demersal fish community in Malaysia. *In* Proceedings of the first Asian fisheries forum (J. L. Maclean, L. B. Dizon, and L. V. Hosillows, eds.), p. 349–352. Asian Fisheries Society, Manila, Philippines.

Dauwe, B.
1993. Ecology of the seahorse *Hippocampus reidi* on the Bonaire coral reef (N.A.): habitat, reproduction and community interaction. M.S. thesis, 65 p. Rijksuniversiteit, Groningen, Netherlands.

deBruyn, A. M. H., and J. J. Meeuwig.
2001. Detecting lunar cycles in marine ecology: periodic regression versus categorical ANOVA. Mar. Ecol. Prog. Ser. 214:307–310.

Diamond, S. L., L. G. Cowell, and L. B. Crowder.
2000. Population effects of shrimp trawl bycatch on Atlantic croaker. Can. J. Fish. Aquat. Sci. 57(10):2010–2021.

FAO (Food and Agriculture Organization of the United Nations).
1999. The state of world fisheries and aquaculture 1998, 112 p. FAO, Rome.

Galloway, B. J., and J. G. Cole.
1999. Reduction of juvenile red snapper bycatch in the Gulf of Mexico shrimp trawl fishery. N. Am. J. Fish. Manag. 19(2):342–355.

Graham, G. L.
1995. Finfish bycatch from the Southeastern shrimp fishery. *In* Solving bycatch: considerations for today and tomorrow, p. 115–119. Univ. Alaska Sea Grant College Program Report 96-03, Fairbanks, AK.

Hill, B. J., and T. J. Wassenberg.
1990. Fate of discards from prawn trawlers in Torres Strait. Aust. J. Mar. Freshw. Res. 41:53–64.
2000. The probable fate of discards from prawn trawlers fishing near coral reefs. A study in the northern Great Barrier Reef, Australia. Fish. Res. 48(3):277–286.

Hutchings, P.
1990. Review of the effects of trawling on macrobenthic epifaunal communities. Aust. J. Mar. Freshw. Res. 41: 111–120.

IUCN (International Union for Conservation of Nature and Natural Resources).
1996. World Conservation Congress, 1st session. Resolutions and recommendations. 1.16 Fisheries by-catch; Montreal, Canada, 14–23 October 1996. IUCN, Gland, Switzerland.
2002. 2002 IUCN red list of threatened species. IUCN, Gland, Switzerland and Cambridge, UK. http://www.redlist.org/ [Accessed 21 January 2003].

Jennings, S., and M. J. Kaiser.
1998. The effects of fishing on marine ecosystems. Adv. Mar. Biol. 34:201–352.

Julian, F., and M. Beeson.
1998. Estimates of marine mammal, turtle and seabird mortality for two California gillnet fisheries: 1990–1995. Fish. Bull. 96:271–284.

Kaiser, M. J., and B. E. Spencer.
1995. Survival of by-catch from a beam trawl. Mar. Ecol. Prog. Ser. 126:31–38.

Kvarnemo, C., G. I. Moore, A. G. Jones, W. S. Nelson, and J. C. Avise.
2000. Monogamous pair bonds and mate switching in the Western Australian seahorse *Hippocampus subelongatus*. J. Evol. Biol. 13:882–888.

Larkin, S. L., and R. L. Degner.
2001. The U.S. wholesale market for marine ornamentals. Aquar. Sci. Conserv. 3(1):13–24.

Lourie, S. A., A. C. J. Vincent, and H. J. Hall.
1999. Seahorses: an identification guide to the world's species and their conservation, 214 p. Project Seahorse, London, UK.

Mensink, B. P., C. V. Fischer, G. C. Cadee, M. Fonds, C. C. Ten Hallers-Tjabbes, and J. P. Boon.
2000. Shell damage and mortality in the common whelk *Buccinum undatum* caused by beam trawl fishery. J. Sea Res. 43(1):53–64.

Meyer, D. L., M. S. Fonseca, P. L. Murphey, R. H. McMichael, Jr., M. M. Byerly, M. W. LaCroix, P. E. Witfield, and G. W. Thayer.
1999. Effects of live-bait shrimp trawling on seagrass beds and fish bycatch in Tampa Bay, Florida. Fish. Bull. 97:193–199.

Milton, D.
2001. Assessing the susceptibility to fishing of populations of rare trawl bycatch: sea snakes caught by Australia's northern prawn fishery. Biol. Conserv. 101:281–290.

Nijhoff, M.
1993. Reproductive cycle of the seahorse *Hippocampus reidi* on the Bonaire coral reef. M.S. thesis, 49 p. Rijksuniversiteit, Groningen, Netherlands.

Ortiz, M., C. M. Legault, and N. M. Ehrhardt.
2000. An alternative method for estimating bycatch from the U.S. shrimp trawl fishery in the Gulf of Mexico, 1972–1995. Fish. Bull. 98:583–599.

Parrish, J. K.
1999. Using behavior and ecology to exploit schooling fishes. Environ. Biol. Fishes 55:157–181.

Perante, N. C., M. G. Pajaro, J. J. Meeuwig, and A. C. J. Vincent.
2002. Biology of *Hippocampus comes* in the central Philippines. J. Fish Biol. 60(4):821–837.

Perante, N. C., A. C. J. Vincent, and M. G. Pajaro.
1998. Demographics of *Hippocampus comes* seahorses in Bohol, central Philippines. *In* Proceedings of the third international conference on the Marine Biology of the South China Sea; Hong Kong 1996, p. 439–448. Hong Kong Univ. Press, Hong Kong.

Pettovello, A. D.
1999. By-catch in the Patagonian red shrimp (*Pleoticus muelleri*) fishery. Mar. Freshw. Res. 50:123–127.

Pikitch, E. K., J. R. Wallace, E. A. Babcock, D. L. Erickson, M. Saelens, and G. Oddsson.
1998. Pacific halibut bycatch in the Washington, Oregon, and

California groundfish and shrimp trawl fisheries. N. Am. J. Fish. Manag. 18:569–586.

Polacheck, T.

1989. Harbour porpoises and the gillnet fishery: Incidental takes spur population studies. Oceanus 32:63–70.

Probert, P. K., D. G. McKnight, and S. L. Grove.

1997. Benthic invertebrate bycatch from a deep-water trawl fishery, Chatham Rise, New Zealand. Aquatic conservation: marine and freshwater ecosystems 7(1):27–40.

Strawn, K.

1958. Life history of the pygmy seahorse, *Hippocampus zosterae* Jordan and Gilbert, at Cedar Key, Florida. Copeia 1958:16–22.

Tabb, D. C., and N. Kenny.

1969. A brief history of Florida's live bait shrimp fishery with description of fishing gear and methods. FAO Fish. Rep. 57:1119–1134.

Teixeira, R. L., and J. A. Musick.

2000. Reproduction and food habits of the lined seahorse, *Hippocampus erectus* (Teleeostei: Syngnathidae) of Chesapeake Bay, Virginia. Rev. Bras. Biol. 61(1):79–90.

Thrush, S., and P. K. Dayton.

2002. Disturbance to marine benthic habitats by trawling and dredging: implications for marine benthic biodiversity. Ann. Rev. Ecol. Syst. 33:449–473.

Vari, R. P.

1982. Fishes of the western North Atlantic, subfamily Hippocampinae. The seahorses, p. 173–189. Sears Foundation for Marine Research Memoir, vol. 1, Yale Univ., New Haven, CT.

Vincent, A. C. J.

1994. Operational sex ratios in seahorses. Behaviour 128: 153–167.

1995. A role for daily greetings in maintaining seahorse pair bonds. Anim. Behav. 49:258–260.

1996. The international trade in seahorses, vii + 163 p. TRAFFIC International, Cambridge, UK.

Vincent, A. C. J., and L. M. Sadler.

1995. Faithful pair bonds in wild seahorses, *Hippocampus whitei*. Anim. Behav. 50:1557–1569.

Watling, L., and E. A. Norse.

1998. Disturbance of the seabed by mobile fishing gear: a comparison to forest clear cutting. Conserv. Biol. 12(6): 1180–1197.

Weimerskirch, H., N. Brothers, and P. Jouventin.

1997. Population dynamics of wandering albatross *Diomedea exulans* and Amsterdam albatross *D. amsterdamensis* in the Indian Ocean and their relationships with long-line fisheries; conservation implications. Biol. Conserv. 79:257–270.

Zar, J. H.

1996. Biostatistical analysis, 3rd ed., 662 p. Prentice Hall, Upper Saddle River, NJ.

Estimates of survival probabilities for oceanic-stage loggerhead sea turtles (*Caretta caretta*) in the North Atlantic

Karen A. Bjorndal
Alan B. Bolten
Archie Carr Center for Sea Turtle Research and Department of Zoology
University of Florida
P.O. Box 118525
Gainesville, Florida 32611
E-mail address (for K. A. Bjorndal): kab@zoology.ufl.edu

Helen R. Martins
Departamento de Oceanografia e Pescas
Universidade dos Açores
PT-9901-862 Horta
Açores, Portugal

Abstract—Estimates of instantaneous mortality rates (Z) and annual apparent survival probabilities (Φ) were generated from catch-curve analyses for oceanic-stage juvenile loggerheads (*Caretta caretta*) in the waters of the Azores. Two age distributions were analyzed: the "total sample" of 1600 loggerheads primarily captured by sighting and dipnetting from a variety of vessels in the Azores between 1984 and 1995 and the "tuna sample" of 733 loggerheads (a subset of the total sample) captured by sighting and dipnetting from vessels in the commercial tuna fleet in the Azores between 1990 and 1992. Because loggerhead sea turtles begin to emigrate from oceanic to neritic habitats at age 7, the best estimates of instantaneous mortality rate (0.094) and annual survival probability (0.911) not confounded with permanent emigration were generated for age classes 2 through 6. These estimates must be interpreted with caution because of the assumptions upon which catch-curve analyses are based. However, these are the first directly derived estimates of mortality and survival probabilities for oceanic-stage sea turtles. Estimation of survival probabilities was identified as "an immediate and critical requirement" in 2000 by the Turtle Expert Working Group of the U.S. National Marine Fisheries Service.

A major gap in our understanding of sea turtle demography is the level of mortality—both natural and human-induced—experienced by wild populations. Lack of directly derived estimates of mortality (or survival) probabilities for the juvenile oceanic-stage in sea turtle populations is a critical source of uncertainty in development of population models and evaluation of management plans. In current population models, survival estimates for juvenile, oceanic-stage sea turtles are fitted parameters, not directly derived estimates, of survival (Chaloupka, in press; Heppell et al., in press). The Turtle Expert Working Group (2000) identified the estimation of survival probabilities as "an immediate and critical requirement." Population models indicate that survival probability of juvenile oceanic-stage loggerhead sea turtles (*Caretta caretta)* has a substantial effect on overall population growth (Chaloupka, in press; Heppell et al., in press).

Catch-curve analyses have been used for many years to estimate survival probabilities for species harvested in commercial fisheries and, less frequently, for other species (Seber, 1982). Estimates of survival probabilities have been generated from catch-curve analyses for subadult neritic-stage populations of loggerhead sea turtles (Frazer, 1987; Epperly et al., 2001) and Kemp's ridley sea turtles (*Lepidochelys kempii*) (Turtle Expert Working Group, 2000) based on stranding data. Catch-curve analyses confound mortality and permanent emigration, and thus generate estimates of apparent survival probability (Φ),

$$\Phi = S\,(1 - emigration),$$

where S = true survival probability; and
$emigration$ = the probability of permanent emigration.

We estimated survival probabilities (both Φ and S) for juvenile oceanic-stage loggerhead sea turtles in the waters around the Azores, a developmental habitat for the population of loggerhead sea turtles that nest on beaches in the southeastern United States (Bolten et al., 1998). We applied catch-curve analyses to two age distributions of loggerhead sea turtles.

Methods

Two size distributions were compiled for this study. The first ("total sample") comprised 1600 oceanic-stage loggerhead sea turtles that were captured from 1984 through 1995 in the waters of the Azores. Except for a few of the smallest of these sea turtles found as stranded carcasses, they were collected in dipnets after being sighted at the surface of the ocean from the decks of a

Manuscript approved for publication 21 April 2003 by Scientific Editor.
Manuscript received 26 June 2003 at NMFS Scientific Publications Office.
Fish. Bull. 101:732–736 (2003).

variety of vessels. Loggerhead sea turtles captured on longline hooks were excluded from this sample to meet the requirement of equal probability of capture across the age range (see "Results" section). The turtles were measured, tagged, and released soon after capture. The juvenile loggerhead sea turtles ranged in size from 8.5 to 82.0 cm curved carapace length (mean=33.1 cm, SD=11.6) measured from the anterior point at midline to the posterior notch at midline between the supracaudals (Bolten, 1999). For 248 turtles, straight-line carapace measurements were converted to curved carapace length, as described in Bjorndal et al. (2000).

The second age distribution ("tuna sample") was a subset of the total sample and comprised 733 loggerhead sea turtles captured by crews of commercial tuna vessels in the Azores between 1990 and 1992. We analyzed the tuna sample in addition to the total sample because the tuna sample was collected over a shorter interval (1990–92) than was the total sample (1984–95). In addition, sizes of vessels from which turtles were captured were more consistent for the tuna sample. This collaborative project with the tuna fleet is described in Bolten et al. (1993); sea turtles are not bycatch in the tuna fishery. Sea turtles were sighted at the surface while the crews were scanning for indications of tuna feeding activity. The turtles were then captured in dipnets, tagged and measured by a crew member, and released at sea. The juvenile loggerhead sea turtles ranged from 11.0 to 82.0 cm curved carapace length (mean=33.5, SD=11.2). No conversion from straight to curved measurements was required.

The size distributions were converted to age distributions by using a size-at-age function developed for this population based on a skeletochronological study (Table 1; Bjorndal et al., 2003). Catch curves were generated for each age distribution by plotting the natural log of N_x against x, where N_x is the number of turtles of age x. The catch curves were truncated by excluding age classes with fewer than five individuals, as recommended by Seber (1982). The age at which the population fully recruited to the capture method (threshold age) was identified as the age with the highest $\ln(N_x)$. Linear regression analyses of the values on the right-hand or declining slope of the distribution were used to generate estimates of total instantaneous mortality rate (Z), which is expressed on an annual basis and is the absolute value of the slope of the regression line. Annual apparent survival probability (Φ) was estimated as e^{-Z}. The statistical software S-PLUS (Guide to statistics, vol. 1, MathSoft, Seattle, WA) was used for regression analyses.

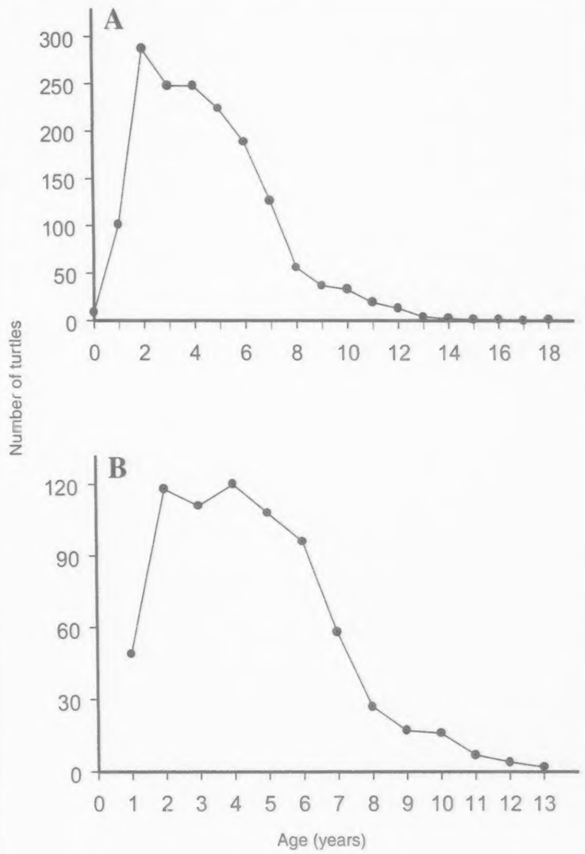


Figure 1

Age distributions of (**A**) total sample (n=1600) and (**B**) tuna sample (n=733).

Results

The age distributions for the two samples are shown in Figure 1 and the catch curves in Figure 2. For catch-curve analyses, the age distributions were truncated to the 12-year age class for the total sample and to the 11-year age class for the tuna sample because older age classes contained fewer than 5 individuals (Seber, 1982). The selection of the appropriate threshold age—the first fully recruited age class to the capture method—can be difficult but is critical for the analyses (Seber, 1982; Isaac, 1990). We designated 2 years as the threshold age for the total sample and 4 years for the tuna sample; in the tuna sample, the 4-year age class was slightly larger than the 2-year age class (120 vs. 118 turtles, Table 1).

We believe that turtles between ages 2 and 12 in the total sample and between 4 and 11 in the tuna sample meet the assumption of equal capture probability. With increase in turtle size, capture probability is a compromise; greater visibility of larger turtles is countered by greater difficulty in capturing larger turtles. Loggerhead sea turtles captured incidentally by longline vessels were excluded from our sample because there is a capture bias toward the largest size classes. The mean size of the turtles in the total sample

(33.1 cm) was significantly different (Kolmogorov-Smirnov test, $ks=0.6966$, $P<0.001$) from the mean size of loggerhead sea turtles caught in longline fisheries in the Azores during an experiment conducted in 2000 (49.8 cm; $n=224$; Bolten, in press).

The catch curves for both samples (Fig. 2) reveal a change in slope at age 6. The 7-year age class includes loggerhead sea turtles with curved carapace lengths >46 cm—the size at which they begin to leave oceanic habitats and recruit to neritic habitats (Bjorndal et al., 2000). Thus, the sharper decline beginning with age 7 reflects the migration of turtles out of the sampling area. Estimates of total instantaneous mortality rate (Z) and annual apparent survival probability (Φ) were generated for three age ranges for each sample: from threshold age to final age, from threshold age to pre-emigration age (6 years), and from pre-emigration age to final age. For the total sample, the age ranges were 2 to 12 years, 2 to 6 years, and 6 to 12 years, respectively; for the tuna sample, the ages were 4 to 11, 4 to 6, and 6 to 11 years (Fig. 2, Table 2).

Discussion

Estimates of mortality and survival generated from catch curves should be interpreted with caution for several reasons. First, the analysis assumes a stable age distribution, which we cannot confirm for North Atlantic loggerhead sea turtles. Second, the analysis assumes that mortality rates were consistent over the years of the study. The similarity of the mortality and survival estimates between the total sample (1984–95) and the tuna sample (1990–92) suggests that the estimates for the total sample are not greatly affected by heterogeneity among years. Third, converting size distributions to age distributions based on a size-at-age function introduces some level of error. We believe the error from our size-at-age function is small, as discussed in Bjorndal et al. (in press).

Fourth, the analysis assumes no size or age effect on mortality rates. The catch curves for both the total sample and tuna sample reveal a size or age effect with a pivot point at age 6. This size or age effect reflects the beginning of emigration out of our study area. Loggerhead sea turtles begin to leave oceanic habitats around the Azores and recruit to neritic habitats at 7 years of age, at ~46 cm curved carapace length (Bjorndal et al., 2000, 2003). This change in slope demonstrates the fifth difficulty in interpreting catch-curve estimates: permanent emigration and mortality are confounded in the estimates. That is, declines in numbers with age, whether they are due to emigration or mortality, are included in the estimate of mortality. The confounding of emigration and mortality can introduce a major error in estimates of mortality in populations—such as sea turtle populations in oceanic and neritic habitats—that undergo developmental migrations.

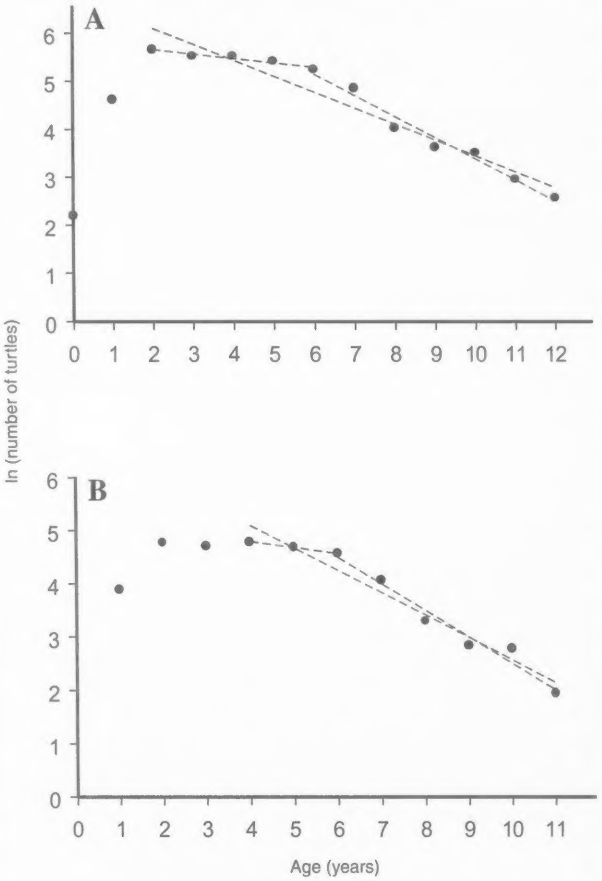


Figure 2

Catch curves for (**A**) total sample and (**B**) tuna sample. Dashed lines are linear regressions for entire sample, threshold age to age 6, and age 6 to final age (see Table 2 for regression statistics).

Because little permanent emigration apparently occurs before the age of 7, the survival estimates for the ages prior to 7 years are our best estimates of true survival (S). As can be seen in Table 2, the estimates of total instantaneous mortality and annual survival are similar for the two samples. We believe that the estimate of S (0.911 and 0.894, respectively, for the total sample and tuna sample) would apply to the entire life stage over the size range from 20 to 65 cm CCL for most sources of mortality other than fisheries biased to large sizes, such as longline fisheries. For predation, as sea turtles increase in size, they outgrow the prey size of some fish predators, but they also grow into the prey size of the largest predators, such as killer whales and humans (although the latter source is now very low in the Azores as a result of legislation and education [senior author, personal obs.]). Death from ingestion of or entanglement in marine debris would probably not vary substantially over this size range. However, mortality from inciden-

Table 1

Size ranges of age classes and age distributions for total sample and tuna sample. CCL is curved carapace length, N_x is the number of turtles in each age class x, and YOY is young of year.

Size range (cm CCL)	Age	Total sample		Tuna sample	
		N_x	$\ln(N_x)$	N_x	$\ln(N_x)$
< 15.0	YOY	9	2.197	—	—
15.0–20.5	1	101	4.615	49	3.892
20.5–26.1	2	287	5.659	118	4.771
26.1–31.7	3	248	5.513	111	4.710
31.7–36.9	4	248	5.513	120	4.787
36.9–41.7	5	224	5.412	108	4.682
41.7–46.5	6	189	5.242	96	4.564
46.5–49.9	7	127	4.844	58	4.060
49.9–52.3	8	56	4.025	27	3.296
52.3–55.0	9	37	3.611	17	2.833
55.0–58.2	10	33	3.497	16	2.773
58.2–61.6	11	19	2.944	7	1.946
61.6–65.0	12	13	2.565	—	—

tal capture in longline fisheries in the Azores does increase with size, with the 2 to 6 year age classes experiencing very little mortality (Bolten, in press). Thus, if our estimate of S (calculated for the age classes between threshold age and age 6) were applied to the entire oceanic stage, the effect of mortality in longline fisheries, or other fisheries biased to large size classes, would be underestimated.

The estimate of 0.911 for annual survival probabilities for oceanic-stage loggerhead sea turtles in the waters of the Azores indicates high survival in this lifestage without mortality from longline fisheries. Species characterized by long life and late sexual maturity, such as loggerhead sea turtles, require very high survival throughout immature stages to maintain populations (Congdon et al., 1993; Crouse, 1999). This high probability of survival is also consistent with the theory that lower predation in oceanic habitats compared to neritic habitats is the selective pressure that maintains oceanic juvenile stages in most species of sea turtles (Bolten, 2003).

Table 2

Estimates of instantaneous mortality rates (Z) and annual apparent survival probabilities (Φ, estimated as e^{-Z}) for oceanic-stage loggerheads in the waters of the Azores generated from catch-curve analyses. r^2 (coefficient of determination) and P values are from linear regression analyses (see Fig. 2).

Age range (years)	Z	Φ	r^2	P
Total sample				
2 to 12	0.333	0.720	0.935	< 0.001
2 to 6	0.094	0.911	0.923	= 0.009
6 to 12	0.441	0.643	0.974	< 0.001
Tuna sample				
4 to 11	0.421	0.656	0.954	< 0.001
4 to 6	0.112	0.894	0.999	= 0.021
6 to 11	0.498	0.608	0.966	< 0.001

In two updated matrix models for North Atlantic loggerhead sea turtles (that differed in stage lengths), Heppell et al. (in press) derived fitted estimates of 0.745 and 0.875 for annual survival probabilities of the oceanic stage, which they defined as spanning 5 to 45 cm carapace length. Chaloupka (in press) derived an estimate of annual survival probability for oceanic-stage loggerhead sea turtles in Australia of 0.67 sampled from a logistic probability density function that ranged from 0.60 to 0.76 and had a mode at 0.67. The tuned estimate of 0.67 was derived from a stochastic simulation model that incorporated empirically based survival probability estimates for all age classes in the model except the oceanic phase (Chaloupka and Limpus, 2002; Chaloupka, in press). The estimate of 0.67 was generated for a size range from posthatchlings that have left the waters directly adjacent to the nesting beach to subadults that begin to leave the oceanic habitats at a size of 69 cm curved carapace length (Chaloupka and Limpus, 2002).

The fitted estimates for annual survival from the Heppell et al. (in press) models and the Chaloupka (in press) model are lower than the estimates in our study, but the size ranges are different. In the Heppell et al. (in press) models and the Chaloupka (in press) model, the oceanic stage includes the posthatchling phase during which loggerhead sea turtles migrate from nesting beaches to their oceanic habitats. We could not include this early posthatchling phase in our estimates of survival of oceanic-stage loggerhead sea turtles in the waters of the Azores because many turtles in this phase have not reached the Azores and they are younger than our threshold ages. We believe that mortality in this early transitional stage when loggerhead sea turtles first cross the Atlantic may be high. In addition to high rates of predation, winds and currents can overwhelm the swimming and orientation abilities of the posthatchling sea turtles, transporting the turtles to habitats, such as waters off the British Isles, that cannot sustain them (Carr, 1986; Hays and Marsh, 1997). Generating directly derived estimates of survival probabilities of loggerhead sea turtles younger than 2 years of age should be a high priority.

Acknowledgments

This study would not have been possible without the support of our colleagues in the Azores: "Equipa Tartaruga" at the Department of Oceanography and Fisheries (DOP), University of the Azores; the captains and crews of the commercial tuna fleet based in Horta and Pico; and J. and G. Franck of the MY *Shanghai*. We thank M. Chaloupka for encouragement to pursue catch-curve analysis. We thank M. Chaloupka and J. Seminoff for comments on earlier drafts of the manuscript and P. Eliazar for technical assistance. This project was funded by the U.S. National Marine Fisheries Service and the Disney Wildlife Conservation Fund. All work was conducted in compliance with the Institutional Animal Care and Use Committee, University of Florida.

Literature cited

Bjorndal, K. A., A. B. Bolten, T. Dellinger, C. Delgado, and H. R. Martins.
2003. Compensatory growth in oceanic loggerhead sea turtles: response to a stochastic environment. Ecology 84:1237–1249.

Bjorndal, K. A., A. B. Bolten, and H. R. Martins.
2000. Somatic growth model of juvenile loggerhead sea turtles *Caretta caretta*: duration of pelagic stage. Mar. Ecol. Prog. Ser. 202:265–272.

Bolten, A. B.
1999. Techniques for measuring sea turtles. *In* Research and management techniques for the conservation of sea turtles (K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly, eds.), p. 110–114. IUCN/SSC Marine Turtle Specialist Group Publication 4, Washington, DC.
2003. Variation in sea turtle life history patterns: neritic versus oceanic developmental stages. *In* Biology of sea turtles, vol. 2 (P. L. Lutz, J. A. Musick, and J. Wyneken, eds.), p. 243–257. CRC Press, Boca Raton, FL.
In press. Active swimmers—passive drifters: the oceanic juvenile stage of loggerheads in the Atlantic system. *In* Loggerhead sea turtles (A. B. Bolten and B. E. Witherington, eds.). Smithsonian Institution Press, Washington, DC.

Bolten, A. B., K. A. Bjorndal, H. R. Martins, T. Dellinger, M. J. Biscoito, S. E. Encalada, and B. W. Bowen.
1998. Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analysis. Ecol. Appl. 8:1–7.

Bolten, A. B., H. R. Martins, K. A. Bjorndal, and J. Gordon.
1993. Size distribution of pelagic-stage loggerhead sea turtles (*Caretta caretta*) in the waters around the Azores and Madeira. Arquipelago 11A:49–54.

Carr, A.
1986. Rips, FADS, and little loggerheads. BioScience 36: 92–100.

Chaloupka, M.
In press. Simulation modeling of population viability for loggerhead sea turtles exposed to competing mortality risks in the western south Pacific region. *In* Loggerhead sea turtles (A. B. Bolten and B. E. Witherington, eds.). Smithsonian Institution Press, Washington, DC.

Chaloupka, M. Y., and C. J. Limpus.
2002. Survival probability estimates for the endangered loggerhead sea turtle resident in southern Great Barrier Reef waters. Mar. Biol. 140:267–277.

Congdon, J. D., A. E. Dunham, and R. C. van Loben Sels.
1993. Delayed sexual maturity and demographics of Blanding's turtle (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. Conserv. Biol. 7:826–833.

Crouse, D. T.
1999. The consequences of delayed maturity in a human-dominated world. Am. Fish. Soc. Symp. 23:195–202.

Epperly, S. P., M. L. Snover, J. Braun-McNeill, W. N. Witzell, C. A. Brown, L. A. Csuzdi, W. G. Teas, L. B. Crowder, and R. A. Myers.
2001. Stock assessment of loggerhead sea turtles of the western North Atlantic. *In* Stock assessments of loggerhead and leatherback sea turtles and an assessment of the impact of the pelagic longline fishery on the loggerhead and leatherback sea turtles of the western North Atlantic, p. 3–66. NOAA Tech. Memo. NMFS-SEFSC-455.

Frazer, N. B.
1987. Preliminary estimates of survivorship for wild juvenile loggerhead sea turtles (*Caretta caretta*). J. Herpetol. 21:232–235.

Hays, G. C., and R. Marsh.
1997. Estimating the age of juvenile loggerhead sea turtles in the North Atlantic. Can. J. Zool. 75:40–46.

Heppell, S. S., L. B. Crowder, D. T. Crouse, S. P. Epperly, and N. B. Frazer.
In press. Population models for Atlantic loggerheads: past, present and future. *In* Loggerhead sea turtles (A. B. Bolten and B. E. Witherington, eds.). Smithsonian Institution Press, Washington, DC.

Isaac, V. J.
1990. The accuracy of some length-based methods for fish population studies, 81 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Seber, G. A. F.
1982. The estimation of animal abundance and related parameters, 654 p. Macmillan Publishing Co., New York, NY.

Turtle Expert Working Group.
2000. Assessment update for the Kemp's ridley and loggerhead sea turtle populations in the western North Atlantic. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-SEFSC-444, 115 p.

Developing a growth-transition matrix for the stock assessment of the green sea urchin (*Strongylocentrotus droebachiensis*) off Maine

Yong Chen
School of Marine Sciences
218 Libby Hall
University of Maine
Orono, Maine 04469
E-mail address: ychen@maine.edu

Margaret Hunter
Maine Department of Marine Resources
P.O. Box 8
West Boothbay Harbor, Maine 04575

Robert Vadas
Department of Biological Sciences
University of Maine
Orono, Maine 04469

Brian Beal
Division of Environmental and Biological Sciences
University of Maine
Machias, Maine 04654

Abstract—The green sea urchin (*Strongylocentrotus droebachiensis*) is important to the economy of Maine. It is the state's fourth largest fishery by value. The fishery has experienced a continuous decline in landings since 1992 because of decreasing stock abundance. Because determining the age of sea urchins is often difficult, a formal stock assessment demands the development of a size-structured population dynamic model. One of the most important components in a size-structured model is a growth-transition matrix. We developed an approach for estimating the growth-transition matrix using von Bertalanffy growth parameters estimated in previous studies of the green sea urchin off Maine. This approach explicitly considers size-specific variations associated with yearly growth increments for these urchins. The proposed growth-transition matrix can be updated readily with new information on growth, which is important because changes in stock abundance and the ecosystem will likely result in changes in sea urchin key life history parameters including growth. This growth-transition matrix can be readily incorporated into the size-structured stock assessment model that has been developed for assessing the green sea urchin stock off Maine.

Manuscript approved for publication 17 April 2003 by Scientific Editor.

Manuscript received 26 June 2003 at NMFS Scientific Publications Office.

Fish Bull. 101:737–744 (2003).

The green sea urchin (*Strongylocentrotus droebachiensis*) fishery is the state's fourth largest fishery by value, worth $20.3 million to harvesters in 1999. The fishery is managed by limited entry, a limited number of opportunity dates that are established each year by recommendation of the sea urchin zone council (SUZC), and minimum and maximum size limits. The fishery is further regulated seasonally by two zones that correspond to variation in spawning time along the coast (Vadas et al., 1997).

The Maine sea urchin fishery began in the late 1980s and reached its peak in landings in 1992. It has since experienced a continuous decline in landings, mainly resulting from large decreases in sea urchin stock abundance (Fig. 1). Although the large decrease in abundance is evident in many studies (Steneck and Vadas[1]; Harris[2]) and apparent to the sea urchin fishing industry, the catch-per-unit-of-effort (CPUE) data derived from the fishery have shown no significant decreases over the last 10 years (Fig. 1). We need to perform a formal stock assessment to better understand the population dynamics of the sea urchin stock and to develop an optimal management strategy.

A population dynamics model for the sea urchin stock should provide reliable estimates of model parameters with suitable statistical methods (Hilborn and Walters, 1992; Chen and Paloheimo, 1998; Walters, 1998). A size-structured population dynamics model is needed for the sea urchin fishery because sea urchins are difficult to age and growth varies widely among individuals (Quinn and Deriso, 1999).

One of the key components of a size-structured population dynamics model is a growth-transition matrix, which describes the probability of an organism growing from one size class to another size class in a given unit of time (Sullivan et al., 1990; Sullivan, 1992). In practice, two approaches can be used to incorporate a growth-transition matrix into a stock assessment: one is to incorporate the growth-transition matrix and simultaneously estimate matrix parameters with parameters that describe other biological processes in the fishery (Sullivan et al., 1990), and the other approach is to estimate the growth-transition matrix independent of other stock assessment models (Chen et al., 2000). The former considers covariance among different processes by estimating all parameters simultaneously, but makes the analysis more complicated. The latter approach reduces the complexity of modeling but does not consider the covariance of growth and other biological processes. Because size-structured models are often complicated and have many parameters to be estimated, the estimation of a growth-transition matrix outside the main modeling process may be preferable (Chen et al., 2000). In either case, the quality of the growth-transition matrix can greatly influence the quality of the stock assessment. It is thus essential to develop a growth-transition matrix for the Maine sea urchin stock that can capture the variations in growth increments among individuals.

---

[1] Steneck, R., and R. L. Vadas. 2002. Personal commun. School of Marine Sciences, University of Maine, Orono, ME 04469.

[2] Harris, L. 2002. Personal commun. Department of Zoology, University of New Hampshire, Durham, NH 03824.

The information required in estimating a growth-transition matrix includes the mean growth increment in a given unit of time and its associated variation for sea urchins of different sizes. Because growth rates of sea urchins vary with size, growth increments also vary with size, and this variation in growth with size is rarely constant. This has been implicit in the statements of model assumptions in many papers (e.g. Sullivan et al., 1990; Sullivan, 1992, Quinn and Deriso, 1999). However, because the variance for growth increments is difficult to estimate, it is often assumed to be constant for organisms of different sizes (Quinn and Deriso, 1999). Such an assumption of constant variation in growth increment is rather unrealistic and may introduce biases in estimating a growth-transition matrix. Thus, for the Maine sea urchin we need to develop an approach that can explicitly consider nonconstant variances for growth increments of sea urchins of different sizes.

Growth of the sea urchin along the Maine coast has not been studied extensively and the data are limited. The data we used for this study were from Vadas et al. (2002) who collected size-at-age data on sea urchins in two habitats (barren and kelp) from three areas along the coast of Maine.

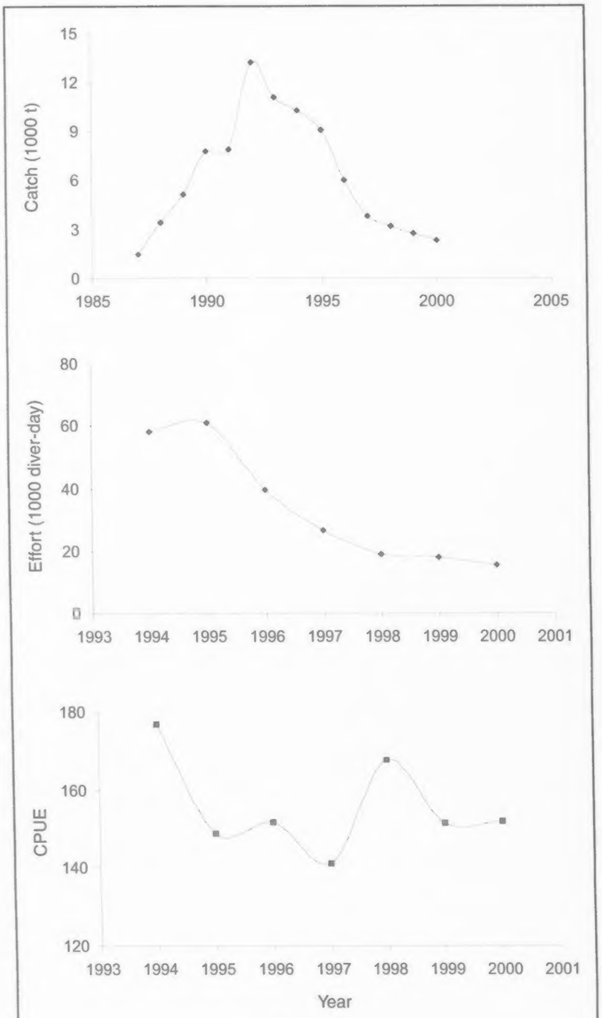


Figure 1

Observed catch measured in metric tons, effort measured in diver-hours, and catch per unit of effort measured in pounds per diver-hour for the sea urchin fishery in management zone 2 in Maine. Zone 1 has a similar temporal pattern.

Methods and materials

Previous studies have indicated that many environmental variables might influence the growth of the sea urchin (Meidel and Scheibling, 1998; Russell, 1998). Sea urchins in favorable habitats, feeding on preferred seaweeds, grow faster than those feeding on less favorable algae and mussels, and sea urchins on barren grounds grow slower. Even in the same habitat, different rates of growth were identified (Vadas, 1977). Previous studies divided the coast of Maine into three regions, northeast, center, and southwest (Vadas et al., 1997). For each region, sea urchin samples were randomly taken from two habitats, barren and kelp. Size-at-age data were collected in 1997–98 for sea urchins in each habitat and area (Vadas and Beal[3]). Detailed descriptions about the derivation of size and age information, justification for dividing the Maine coast, and selection of the habitats can be found in Vadas et al. (1997) and Vadas et al. (2002).

---

[3] Vadas, R. L., and B. F. Beal. 1999. Temporal and special variability in the relationships between adult size, maturity and fecundity in green sea urchins: the potential use of a roe-yield standard as a conservation tool. Report to the Maine Department of Marine Resources, Augusta, Maine 04333.

Vadas et al. (1997) modeled the size-at-age data using the von Bertalanffy growth function (VBGF) described as

$$L_t = L_\infty (1 - e^{-K(t - t_0)}), \tag{1}$$

where L_t = size at age t;
L_∞ = defined as the mean asymptotic length that the sea urchin may attain;
K = the Brody growth parameter; and
t_0 = the hypothetical age of size 0 (Ricker, 1975).

For each area and habitat, a VBGF was used to fit the size-at-age data. Three parameters in the VBGF (i.e. L_∞, K, and t_0) and their standard errors were estimated by using the nonlinear least squares method. These estimates were presented in Vadas and Beal[3] and Vadas et al. (2002), and were made available to the authors of the present study (Table 1). Clearly there were large differences in the estimates of L_∞ and K and their associated variations among different areas and habitats (Table 1).

The L_∞'s estimated for different areas and habitats ranged from 63.1 (northeast region with barren habitat) to 95.2 mm (southeast region with kelp habitat) (Table 1) and tended to be smaller than some large individuals observed in the fishery (about 100 mm, Vadas, 1977; Hunter, unpubl. data). This likely resulted from relatively small sample sizes that covered relatively small areas, in a relatively short period, compared with the fishery catch, which targeted larger-size individuals. The exclusion of individuals in the fishery catch that were larger than the L_∞'s estimated in Vadas and Beal[3] and Vadas et al. (2002) from the calculation of the growth-transition matrix may underestimate the variability in sea urchin growth, thus introducing errors in stock assessment. Based on the data collected in the Maine sea urchin fishery (Hunter, unpubl. data) and previous studies (Vadas, 1977), 100 mm was considered a reasonable value for the average asymptotic size (L_∞) for sea urchins on the coast of Maine. However, more extensive sampling needs to be done in the future to verify this estimate.

Table 1

The average asymptotic size (L_∞) and Broady growth coefficient (K) estimated for different areas and habitats along the coast of Maine in the study done by Vadas et al. (1997, 2002). Coefficient of variation (CV) was calculated by using Equation 2.

		Parameter		Coefficient of variation (CV)	
Area	Habitat	L_∞	K	CV(L_∞)	CV(K)
Northeast	Barren	63.1	0.1404	0.242	1.209
Northeast	Kelp	88.5	0.1263	0.224	0.543
Center	Barren	67.0	0.2315	0.084	0.354
Center	Kelp	63.4	0.3268	0.065	0.248
Southeast	Barren	80.1	0.1776	0.099	0.397
Southeast	Kelp	95.2	0.1181	0.128	0.338

We might be able to derive an estimate of L_∞ for the Maine sea urchin stock based on the examination of the data collected from the fishery and other studies (Ricker, 1975; Moreau, 1987; Chen et al., 1992). An estimate of K for the whole Maine urchin stock is, however, more difficult because K is an abstract rate describing how fast organisms approach the L_∞ and there are no observations or background information with which to compare estimates (Ricker, 1975; Moreau, 1987). We thus need to develop an approach to estimate K for the Maine sea urchin stock which corresponds to the value we assumed for the L_∞. Many studies have indicated that estimates of K and L_∞ tend to be highly and negatively correlated (e.g. Moreau, 1987; Chen and Harvey, 1994). Thus, a fish population or species with a large L_∞ tends to have a low K value, and *vice versa* (Gallucci and Quinn, 1979; Chen et al., 1992). This suggests a strong relationship between L_∞ and K estimates (Pauly, 1980; Stergiou, 1993). Such a relationship may be used to estimate K for a given L_∞ or to estimate L_∞ for a given K. In this study we developed and used the following empirical approach to derive K for a given value of L_∞ and its associated uncertainties in the development of a growth-transition matrix: 1) conduct a regression analysis for K and L_∞ estimated for different areas and habitats along the coast of Maine (Table 1); 2) calculate coefficients of variation (CV) for each K and L_∞ (Table 1) as

$$\begin{aligned} CV(K) &= \frac{\text{standard error for } K}{K}, \text{ and} \\ CV(L_\infty) &= \frac{\text{standard error for } L_\infty}{L_\infty}, \end{aligned} \tag{2}$$

and conduct a regression analysis of CV(K) and CV(L_∞) estimates of different areas and habitats (data in Table 1); 3) use 100 mm to approximate L_∞ and use this L_∞ to estimate K from the regression analysis between K and L_∞; and 4) calculate the average CV for L_∞'s of different areas and habitats and then use the average CV(L_∞) to estimate CV(K) from the CV(K)–CV(L_∞) regression equation.

Because K and L_∞ were estimated for different areas and habitats and had different precisions, outliers might arise in the regression analyses. To avoid possible bias introduced by outliers, we used a reweighted least squares (RLS) method for the regression analyses (Chen et al., 1994). This method involves conducting a robust least median of squares (LMS) analysis to identify outliers (Rousseeuw and Leroy, 1987) and justifying the identified outliers by using background information, followed by a weighted LS analysis where justified outliers are weighted by 0 and other data have a weight of 1 (Chen et al., 1994). In the two regression analyses (i.e. steps 1 and 2), L_∞ and CV(L_∞) were used as the independent variables and K and CV(K) were used as the dependent variables. The reason for this choice (instead of the other way around) is that L_∞ is often estimated more reliably and with much smaller variations (Chen et al., 1992; also see Table 1), whereas K is often less reliably estimated (Moreau, 1987). One of the basic assumptions for a regression analysis is that the independent variable is error free. In practice, this assumption is often relaxed when the independent variable has a much smaller error than the dependent variable (McArdle, 1988). The violation of the normal distribution assumption for the errors in the regression analyses may bias the test for the significance of the regression model and its parameters using common parametric tests (F- or t-tests), but does not necessarily result in biases in the regression analysis (Sen and Srivastava, 1990)."

Given K and L_∞, the growth increment during a unit of time (i.e. year) can be calculated as

$$\Delta L_n = (L_\infty - L_n)(1 - e^{-K}), \tag{3}$$

where K and L_∞ are the true values without errors; n indexes size class; and L_n is the middle point of the n^{th} size class. With Equation 3, we can develop two approaches to estimate the growth-transition matrix. One approach is a Monte Carlo simulation. We can randomly sample H sets of K and L_∞ values from their joint distributions (thus consider their covariance) and then use them in Equation 3 to calculate H sets of ΔL for each size group. We can then derive the probability distribution for ΔL from these H sets of ΔL values for each size group. The Monte Carlo simulation approach is straightforward but requires extensive calculations, in particular when there are a large number of size groups. It is also inconvenient to update the growth-transition matrix when there are new growth data or large changes in growth due to changes in the environment. The second approach is analytic and not so straightforward, but it is easy to update with new information and is less computationally intensive. It is likely that the growth-transition matrix for the Maine sea urchin fishery will need to be updated because of possible changes in growth caused by changes in the sea urchin population size and its ecosystem. Thus we used the second approach, which is described as follows.

Assuming the uncertainties associated with the VBGF parameters L_∞ and K are ΔL_∞ and ΔK respectively, where, $\Delta L_\infty \in N(0, \sigma^2_{L_\infty})$ and $\Delta K \in N(0, \sigma^2_K)$, we have

$$L_\infty = \overline{L}_\infty + \Delta L_\infty \text{ and } K = \overline{K} + \Delta K, \tag{4}$$

where $\overline{L}_\infty$ and $\overline{K}_\infty$ are the estimated parameters. Replacing the true values of L_∞ and K in Equation 3 with Equation 4 and using the approximation $e^{\Delta X} \approx 1 + \Delta X$ for small ΔX, we have

$$\begin{gathered} \Delta L_n \approx (\overline{L}_\infty - L_n)(1 - e^{-\overline{K}}) + \\ \left[\Delta L_\infty (1 - e^{-\overline{K}}) - (\overline{L}_\infty - L_n)\Delta K e^{-\overline{K}} - \Delta L_\infty \Delta K e^{-\overline{K}}\right] = \overline{\Delta L_n} + \varepsilon_n, \end{gathered} \tag{5}$$

where

$$\overline{\Delta L_n} = (\overline{L}_\infty - L_n)(1 - e^{-\overline{K}}) \tag{6}$$

$$\varepsilon_n = \Delta L_\infty (1 - e^{-\overline{K}}) - (\overline{L}_\infty - L_n)\Delta K e^{-\overline{K}} - \Delta L_\infty \Delta K e^{-\overline{K}}. \tag{7}$$

Thus, the expected (mean) value of ΔL_n is $\overline{\Delta L}_n$ and variance of ΔL_n can be estimated from Equation 7 as

$$\begin{gathered} \text{Var}(\overline{\Delta L_n}) \approx \sigma^2_{L_\infty}(1 - e^{-\overline{K}})^2 + (\overline{L}_\infty - L_n)^2 \sigma^2_K e^{-2\overline{K}} - \\ 2\text{Cov}(L_\infty, K)(1 - e^{-\overline{K}})(\overline{L}_\infty - L_n)e^{-\overline{K}}. \end{gathered} \tag{8}$$

Items with the order of three and above for ΔL_n and ΔK are omitted in deriving Equation 8 from Equation 7. From Equation 8, it is clear that the variance of the growth increment varies among different size classes.

From $\overline{\Delta L}_n$ estimated in Equation 6, an expected average yearly growth increment was calculated for each size class. The variability for the average yearly growth increment was assumed to follow a normal distribution with a mean of $\overline{\Delta L}_n$ and variance of Var $(\overline{\Delta L}_n)$ estimated from Equation 8. This distribution was used to determine the vector of probabilities of growing from size class k to other size classes. If d_{low} and d_{up} are the lower and upper ends of size class d, the probability of a sea urchin growing from size class n to size class d can be computed as

$$P_{n \to d} = \int_{d_{low}}^{d_{up}} f(x \mid \overline{\Delta L_n}, \text{Var}(\Delta L_n) dx, \tag{9}$$

where x is a random variable having a density probability distribution defined by $f(x \mid \overline{\Delta L_n}, \text{Var}(\Delta L_n))$ with its expected value of $\overline{\Delta L}_k$ and variance of Var$(\overline{\Delta L}_k)$ (Quinn and Deriso, 1999). In the present study we assumed that the x variable was a normal density distribution function with a mean of $\overline{\Delta L}_k$ defined by Equation 6 and with a variance of Var$(\overline{\Delta L}_k)$ defined by Equation 8. The probability of a sea urchin growing from one size to another was estimated for all size classes to form the matrix. Negative growth increments were not permitted. The largest size class acts as a plus group; therefore sea urchins in this group have a probability of 1 of remaining in the group. The model contains 61 size classes, each with 1-mm interval width, ranging from 40 mm in size (midpoint value for size class from 39.5–40.5 mm) to 100 mm.

Because no negative growth was allowed, the summation of the probabilities of a sea urchin of size class k growing into all other size classes was smaller than 1 (because the normal distribution is symmetric). This problem was avoided by standardization which involved dividing the probability of an urchin in a given size class n growing into each size class by the summation of the probabilities of growing from a given size n to all the size classes. All calculations were done in MS-Excel© (Microsoft Office 2000, Microsoft Corporation, Redmond, WA). A worksheet for estimating a growth-transition matrix as described above is available upon request.

Results

The LMS analysis suggested that the logarithmic K and L_∞ data for the barren habitat in the Southwest area was an outlier in the K and L_∞ regression analysis (Fig. 2). The estimated K and L_∞ values for the barren habitat in the Southwest had CVs over 120% and 24%, respectively, much

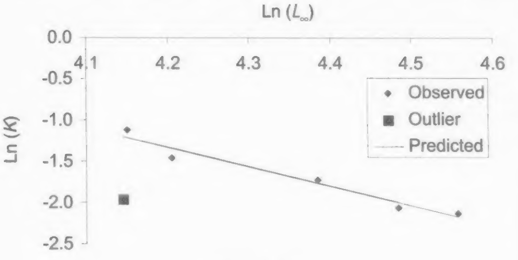


Figure 2

The regression analysis of logarithmic K and L_∞ for different locations and habitats of Maine sea urchins.

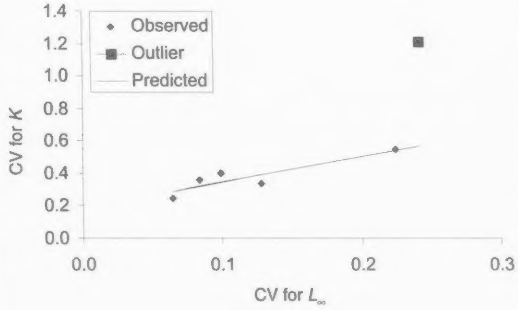


Figure 3

The regression analysis of CVs for K and L_∞ for different locations and habitats of Maine sea urchins.

larger than the estimates for other locations and habitats (Table 1). This was the only site where the K estimate was not significantly different from 0 (thus the VBGF was not significant). We thus concluded that this data point was an outlier because of the poor fit of the VBGF, and subsequently it was given a zero weight in the RLS analysis. The RLS regression equation for K and L_∞ was estimated by

$$Ln(K) = 8.653 - 2.3777\ Ln(L_\infty), \quad P=0.0038, \text{ adj. } r^2=0.94. \tag{10}$$

The standard deviations for the intercept and slope were 1.2605 and 0.28923, respectively. The P value for Equation 10 indicates that the regression model is significant. The adj. r^2 is the coefficient of determination adjusted for the sample size, suggesting 94% of the variance in ln(K) could be explain by the model.

The LMS analysis of the CVs of parameters K and L_∞ also suggested that the barren habitat in the southwest area was an outlier because it had an exceptionally large CV for K (Fig. 3). We thus concluded that this data point was an outlier and should be given a weight of zero in the RLS analysis. The RLS regression equation for the CVs of parameters K and L_∞ was estimated by

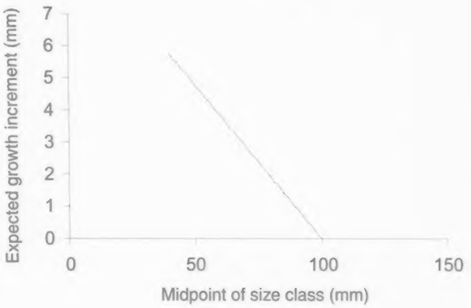


Figure 4

The expected annual growth increment for Maine sea urchins of different size classes.

$$CV(K) = 0.189 + 1.5602\ CV(L_\infty), \quad P=0.034, \text{ adj. } r^2 = 0.76. \tag{11}$$

The standard deviations for the intercept and slope were 0.0561 and 0.42319, respectively. The P value suggested the regression model was significant ($P<0.05$). The value of r^2 suggests 76% of the variance in CV(K) could be explained by the model.

The average CV for L_∞'s of different areas and habitats was 15%. The L_∞ was assumed to have a value of 100 mm in this study as discussed previously. This gave the L_∞ a standard error estimate of 15.0 mm, making its 95% confidence intervals 70 mm to 130 mm. The K value was estimated to be 0.1006 using Equation 10 and L_∞ of 100 mm. Using Equation 11 and the CV for L_∞, the CV for K was estimated to be 42.3%, which yielded the value of 0.0426 for the standard error for K.

The annual expected growth increment decreased quickly with sea urchin size (Fig. 4). The largest expected annual increment was 6 mm for the smallest size class (39.5–40.5 mm) included in the study. The variance for annual growth increments calculated by using Equation 8 was large for small sea urchins. It decreased initially with size, reaching the smallest value at the 59 mm size class (58.5–59.5 mm), followed by a progressive increase with size (Fig. 5). The expected annual growth increment for the largest size class included in this study had the highest variance, which was over eight times as high as the smallest variance (Fig. 5).

The probability distribution of annual growth increment varied among size classes (Fig. 6), reflecting the differences in variances associated with different size classes. The last size class was a plus class, with the probability of staying in the same size class being 1. Figure 6 clearly indicated that no negative growth was allowed.

Discussion

Great variation in growth was observed in the Maine sea urchin stock (Vadas et al., 2002). Such a pattern of variation was reflected in estimating the VBGF parameters for dif-

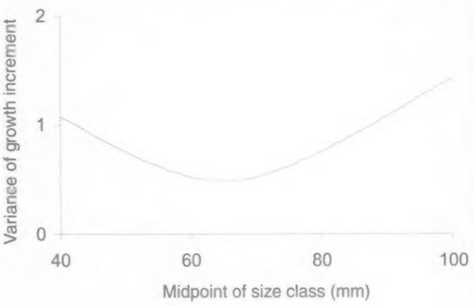


Figure 5

The variances of growth increment estimated for different sea urchin size classes by using Equation 8.

ferent areas and habitats (Table 1). Large standard errors were estimated for the VBGF parameters for sea urchins of the same area and habitat, and large differences occurred in the estimated VBGF parameters between different areas and habitats (Table 1). The approach developed in the present study considered observations made in both the fishery and scientific studies and provided a systematic way to incorporate the large variation in growth into the estimation of a growth-transition matrix, and subsequently into the sea urchin stock assessment.

It should be noted that the algorithm developed for estimating the variance of growth increments is approximate, and violations of the assumptions used in deriving the algorithm may introduce errors in estimating a growth-transition matrix. For example, large errors in estimating K and L_∞ will introduce errors in Equation 5, which was derived by assuming small errors for the two growth parameters. Nonnormal distribution of ΔL with its mean defined by Equation 6 and variance defined by Equation 8 will also result in errors in developing a growth-transition matrix. Other factors that may influence the quality of the estimated growth transition matrix include errors in estimating CVs for K, L_∞ estimated from Equations 10 and 11, and omitting high order items in deriving Equation 8.

Unlike most studies in which the variance for the annual growth increment was assumed to be the same for all size classes (Quinn and Deriso, 1999), our study explicitly suggested that the variance for the annual growth increment changed with size (Fig. 4). The differences in the variance were large between size classes, and changed nonlinearly with size. If a constant variance were used for all size classes, the variance in growth increment would be severely underestimated for large and small fish. This could introduce large biases in a stock assessment.

Size-dependent variation might better describe the variation in annual growth increment. Fish in small size classes tend to grow fast, but their growth tends to be more susceptible to environmental variation than adult growth, often resulting in large variation among individuals (Summerfelt and Hall, 1987). Fish in large size classes (older fish) have to divert some energy to reproduction but tend to have considerable variation in energy allocation strategies among individuals. Differences among adults in the ability to grow can also be considerable because of genetics, specific growth patterns during juvenile stages, and differences in energy allocation between growth and maturation during younger ages (Nikolskii, 1969). This difference may cause large variations in growth for large and old fish (Summerfelt and Hall, 1987; Chen et al., 1988). Compared with old and young ages, growth rates for medium-size and medium-age fish may be less varied (Nikolskii, 1969). This pattern can be reflected realistically in the estimated variation by using the approach derived in our study.

Although the choice of L_∞ was a bit arbitrary in our study, it reflects observations from both the fishery and scientific studies. The largest sea urchins observed in the different scientific studies tend to be smaller than 100 mm, as indicated by the estimated L_∞ values for different areas and habitats (Fig. 1). The inability to observe larger sea urchins in scientific studies may result from relatively small sample sizes, the focus of research (small areas), and the large growth variations even in small spatial scales. The data collected from the fishery were more extensive and covered more areas. This, together with the tendency for taking large individuals in the fishery, may suggest that large individuals are more likely to appear in the fishery, rather than in scientific studies. Thus, it may be reasonable to set the expected value of L_∞ at 100 mm. Also, this higher value corresponds more closely to the upper growth estimates for green sea urchins from the northeast Pacific (Vadas, 1977). The CV was assumed to be 15% for L_∞, resulting in the 95% confidence interval of L_∞ ranging from 70 mm to 130 mm. This range was believed to be a reasonable estimate for the maximum attainable length for green sea urchins on the coast of Maine (Vadas, 1977).

The approach developed in our study can be readily used to incorporate the VBGF parameters estimated from different studies. This can be accomplished by rerunning the regression analyses between K and L_∞ and between CVs for K and L_∞. As more information about the growth of sea urchins on the coast of Maine becomes available, the growth transition matrix can be easily updated to reflect the variation identified in newer studies. The flexibility and ability to easily update and incorporate new information makes this approach desirable to the Maine sea urchin fishery, which is currently undergoing large changes in its population size and has only limited growth data.

The value of 100 mm chosen for L_∞ was rather arbitrary. However, because we considered the negative correlation between K and L_∞ in deriving the growth transition matrix, a small error in the L_∞ estimate would not change the growth-transition matrix greatly. In the future, however, we can conduct a systematic sampling of the stock across its geographical range and derive some forms of weighted average size with a composite variance that captures the range of sizes exhibited by the species. Such an approach would provide us with a better estimate of L_∞.

The growth-transition matrix developed in our study summarizes the growth patterns of sea urchins along the coast of Maine. It can be updated whenever new growth data become available. It can be readily incorporated into

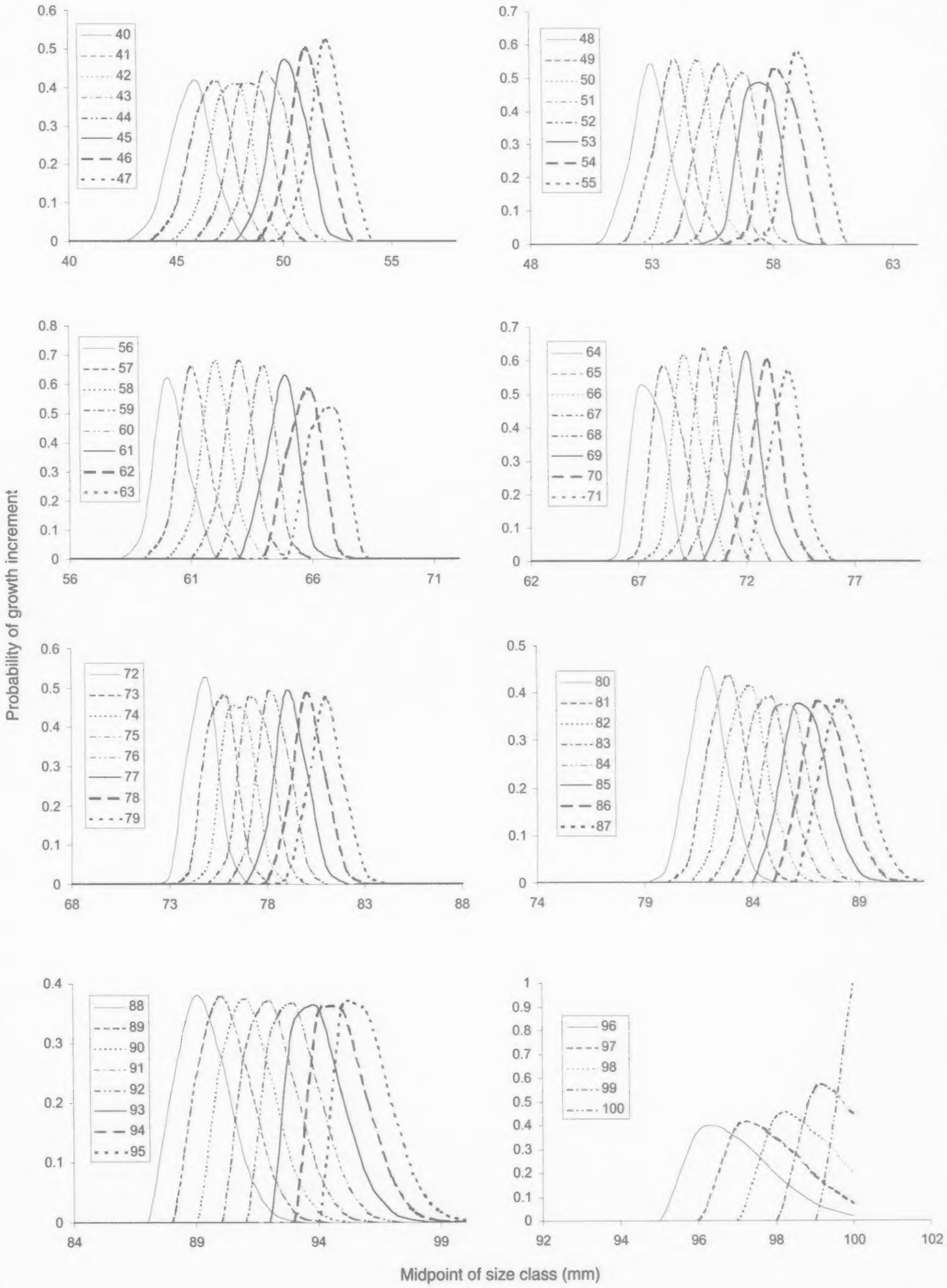


Figure 6

Probabilities of sea urchins growing from one size class to others. Each probability distribution was labeled with the midpoint value of the current size class of the sea urchin.

a size-structured stock assessment model to evaluate the status of sea urchin stock and to evaluate alternative management strategies for the Maine sea urchin fishery (Chen and Hunter, 2003).

Acknowledgments

We would like to thank the Maine Department of Marine Resources, the Northeast Consortium, and the Maine Sea Urchin Zone Council for supporting this study. Constructive and detailed comments from two anonymous reviewers and the scientific editor greatly improved an early version of the manuscript, for which we are grateful.

Literature cited

Chen, S., S. Watanabe, and K. Takagi.
1988. Growth analysis on fish population in the senescence with special reference to an estimation of age at end of reproductive span and life span. Bull. Jpn. Soc. Sci. Fish. 54:1567–1572.

Chen, Y., P. Breen, and N. Andrew.
2000. Impacts of outliers and mis-specification of priors on Bayesian fisheries stock assessment. Can. J. Fish. Aquat. Sci. 57:2293–2305.

Chen, Y., and H. H. Harvey.
1994. Maturation of white sucker, *Catostomus commersoni*, populations in Ontario. Can. J. Fish. Aquat. Sci. 51: 2066–2076.

Chen, Y., and M. Hunter.
2003. Assessing the green sea urchin (*Strongylocentrotus drobachiensis*) stock in Maine, USA. Fish. Res. (Amst.) 60:527–537

Chen, Y., D. A. Jackson, and H. H. Harvey.
1992. A comparison for von Bertalanffy and polynomial functions in modeling fish growth data. Can. J. Fish. Aquat. Sci. 49:1228–1235.

Chen, Y., D. A. Jackson, and J. E. Paloheimo.
1994. Robust regression approach to analyzing fisheries data. Can. J. Fish. Aquat. Sci. 51:1420–1429.

Chen, Y., and J. E. Paloheimo.
1998. Can a more realistic model error structure improve parameter estimation in modelling the dynamics of fish populations? Fish. Res. (Amst.) 38: 9–19.

Gallucci, V. F., and T. J. Quinn II.
1979. Reparameterizing, fitting, and testing a simple growth model. Trans. Am. Fish. Soc. 108:14–25.

Hilborn, R., and C. Walters.
1992. Quantitative fisheries stock assessment: choice, dynamics, and uncertainty, 570 p. Chapman and Hall, New York, NY.

McArdle, B. H.
1988. The structural relationship: regression in biology. Can. J. Zool. 66:2329–2339.

Meidel, S. K., and R. E. Scheibling.
1998. Size and age structure of the sea urchin *Strongylocentrotus droebachiensis* in different habitats. *In* Echinoderms (R. Mooi, M. Telford, eds.), p 737–742. Proceedings of the 9th international echinoderm conference; San Francisco, 5–9 August 1996. A. A. Balkema, Rotterdam, Netherlands.

Moreau, J.
1987. Mathematical and biological expression of growth in fishes: recent trends and further developments. *In* The age and growth of fish (R. C. Summerfelt and G. E. Hall (eds.), p 81–113. Iowa State Univ. Press, Ames, IA.

Nikolskii, G. V.
1969. Theory of fish population dynamics, 323 p. Oliver & Boyd, Edinburgh, UK.

Pauly, D.
1980. On the interrelationships between natural mortality, growth parameters and mean environmental temperature in 175 fish stocks. J. Cons. Int. Explor. Mer 39:175–192.

Quinn, T. J., II, and R. B. Deriso.
1999. Quantitative fish dynamics, 542 p. Oxford Univ. Press, New York, NY.

Ricker, W. E.
1975. Computation and interpretation of biological statistics of fish populations, 382 p. Bull. Fish. Res. Board Can., vol. 191.

Rousseeuw, P. J., and A. M. Leroy.
1987. Robust regression and outlier detection, 352 p. John Wiley & Sons, New York, NY.

Russell, M. P.
1998. Resource allocation plasticity in sea urchin: rapid diet induced, phenotypic changes in the green sea urchin, *Strongylocentrotus droebachiensis* (Müller). J. Exp. Mar. Biol. Ecol. 220:1–14.

Sen, A. K., and M. S. Srivastava.
1990. Regression analysis: theory, methods and applications, 350 p. Springer-Verlag, New York, NY.

Stergiou, K. I.
1993. Nutrient-dependent variation in growth and longevity of the red bandfish, *Cepola macrophthalma* (L.), in the Aegean Sea. J. Fish Biol. 42:633–644.

Sullivan, P. J.
1992. A Kalman filter approach to catch-at-length analysis. Biometrics 48:237–257.

Sullivan, P. J., H. L. Lai, and V. F. Gallucci.
1990. A catch-at-length analysis that incorporates a stochastic model of growth. Can. J. Fish. Aquat. Sci. 47:184–198.

Summerfelt, R. C., and G. E. Hall.
1987. The age and growth of fish, 530 p. Iowa State Univ. Press, Ames, IA.

Vadas, R. L.
1977. Preferential feeding: an optimization strategy in sea urchins. Ecol. Monogr. 47:337–371.

Vadas, R. L., B. Beal, S. Dudgeon, and W. Wright.
1997. Reproductive biology of green sea urchins along the coast of Maine: final report, 59 p. Maine Cooperative Extension Service and Maine Sea Grant Program, Orono, ME.

Vadas, R. L., B. Smith, B. Beal, and T. Dowling.
2002. Sympatric growth morphs and size bimodality in the green sea urchin (*Strongylocentrotus droebachiensis*). Ecol. Monogr. 72:113–132.

Walters, C. J.
1998. Evaluation of quota management policies for developing fisheries. Can. J. Fish. Aquat. Sci. 55:2691–2705.

Reproductive biology of the blue swimmer crab (*Portunus pelagicus*, Decapoda: Portunidae) in five bodies of water on the west coast of Australia

Simon de Lestang

Norman G. Hall

Ian C. Potter

Centre for Fish and Fisheries Research
Division of Science and Engineering
Murdoch University
South Street
Murdoch, Western Australia 6150
E-mail: (for S. de Lestang): simond@murdoch.edu.au

Abstract—*Portunus pelagicus* was collected at regular intervals from two marine embayments and two estuaries on the lower west coast of Australia and from a large embayment located approximately 800 km farther north. The samples were used to obtain data on the reproductive biology of this species in three very different environments. Unlike females, the males show a loosening of the attachment of the abdominal flap to the cephalothorax at a prepubertal rather than a pubertal molt. Males become gonadally mature (spermatophores and seminal fluid present in the medial region of the vas deferentia) at a very similar carapace width (CW) to that at which they achieve morphometric maturity, as reflected by a change in the relative size of the largest cheliped. Logistic curves, derived from the prevalence of mature male *P. pelagicus*, generally had wider confidence limits with morphometric than with gonadal data. This presumably reflects the fact that the morphometric (allometric) method of classifying a male *P. pelagicus* as mature employs probabilities and is thus indirect, whereas gonadal structure allows a mature male to be readily identified. However, the very close correspondence between the CW_{50}'s derived for *P. pelagicus* by the two methods implies that either method can be used for management purposes. *Portunus pelagicus* attained maturity at a significantly greater size in the large embayment than in the four more southern bodies of water, where water temperatures were lower and the densities of crabs and fishing pressure were greater. As a result of the emigration of mature female *P. pelagicus* from estuaries, the CW_{50}'s derived by using the prevalence of mature females in estuaries represent overestimates for those populations as a whole. Estimates of the number of egg batches produced in a spawning season ranged from one in small crabs to three in large crabs. These data, together with the batch fecundities of different size crabs, indicate that the estimated number of eggs produced by *P. pelagicus* during the spawning season ranges from about 78,000 in small crabs (CW=80 mm) to about 1,000,000 in large crabs (CW=180 mm).

Manuscript approved for publication 19 June 2003 by Scientific Editor.

Manuscript received 26 June 2003 at NMFS Scientific Publications Office.

Fish. Bull. 101:745–757 (2003).

Portunid crabs, such as *Portunus pelagicus*, *Scylla serrata*, and *Callinectes sapidus*, form the basis of important commercial and recreational fisheries. The blue swimmer crab (*P. pelagicus*) is found in sheltered nearshore marine waters and estuaries throughout the Indo-West Pacific (Stephenson, 1962; Kailola et al., 1993). In Australia, the commercial catches of this portunid have increased greatly during the last 20 years, and annual catches in 1998 reached 4377 metric tons (t) (Anonymous, 2000). The commercial fishery for *P. pelagicus* in Western Australia is the largest in Australia; the catch in the 1999–2000 financial year weighed 673 t and fetched a wholesale price of approximately $A3 million (CAES[1]).

Large numbers of portunids frequently enter estuaries as juveniles and remain there for an extended period (Hill, 1975; Potter et al., 1983; Perkins-Visser et al., 1996; Potter and de Lestang, 2000). Although female portunids sometimes become ovigerous in estuaries, such individuals emigrate into coastal marine waters, where they release their eggs (Van Engel, 1958; Metcalf et al., 1995; Potter and de Lestang, 2000). In contrast, the individuals of those assemblages of portunids that occupy marine embayments often do not leave these marine environments to spawn and, in cases where there is a salinity gradient, they spawn in the high salinity regions of those systems (e.g. Campbell, 1984; Sumpton et al., 1994; Prager, 1996; Potter and de Lestang, 2000).

The most common method for determining the size at which male crabs attain maturity is to estimate the size at which the pattern of growth of one of its appendages changes from that which characterizes juvenile crabs to that which characterizes adult crabs (e.g. Hartnoll, 1974; Somerton, 1980; Reeby et al., 1990; Muiño et al., 1999). However, this indirect approach is not precise and requires careful measurements of a considerable number of individuals covering a wide size range. Despite the fact that macroscopic characters can be used to distinguish sequential stages in the development of the vas deferentia of portunids (Ryan, 1967a; Meagher, 1971), few studies have attempted to use such staging to determine the body size at which the gonads of male crabs attain maturity (e.g. Reeby et al., 1990). Sumpton et al. (1994) considered that, as in female *P. pelagicus*, a marked loosening of the attachment of the abdominal flap to the cephalothorax signaled the attainment of maturity in male *P. pelagicus*. However, this criterion has yet to be shown to be valid for the males of this species. Although variations in the size at which crustaceans reach maturity among bodies of water and geographical regions may reflect, in part, differences in such features as genetic composition and density, there is a strong overall tendency for the size at maturity of this crustacean to be inversely related to water temperature (Pillai and Nair, 1971; Jones and Simons, 1983; Polovina, 1989; Dugan et al., 1991; Miliou, 1996; Somerton and Donaldson, 1996; Fisher, 1999).

---

[1] CAES (Department of Fisheries, Catch and Effort Statistics). 2002. Unpubl. data. Western Australian Department of Fisheries, Catch and Effort Statistics. Fisheries Western Australia, WA Marine Research Laboratories, West Coast Drive, Waterman, 6020, Perth, Australia.

Estimates of the fecundity of crabs have typically been based on the number of eggs in a single batch of eggs (e.g. Potter et al., 1983; Melville-Smith, 1987; Ingles and Braum, 1989). However, such an approach does not take into account the fact that female crabs often produce more than one batch of eggs during a spawning season (Van Engel, 1958; Pillai and Nair, 1971; Campbell, 1984).

The aims of this study were as follows. 1) Compare the results of three methods directed at determining whether male *P. pelagicus* have attained maturity and elucidate whether each method produces reliable results. 2) Compare aspects of the reproductive biology of *P. pelagicus* in two estuaries and two marine embayments in temperate Australia with those of this species in a large marine embayment in a much warmer and more northern subtropical environment. Particular emphasis will be placed on comparing the size at maturity of both sexes and the periods during which ovigerous females are present, and on proposing reasons for the significance of any differences between the assemblages in these five bodies of water. 3) Use the data collected for one of the marine embayments to determine the age and time of year at which *P. pelagicus* becomes mature and develop a method for deriving the annual fecundity that takes into account the fact that the larger individuals of this species are believed to produce more than one batch of eggs in a spawning season.

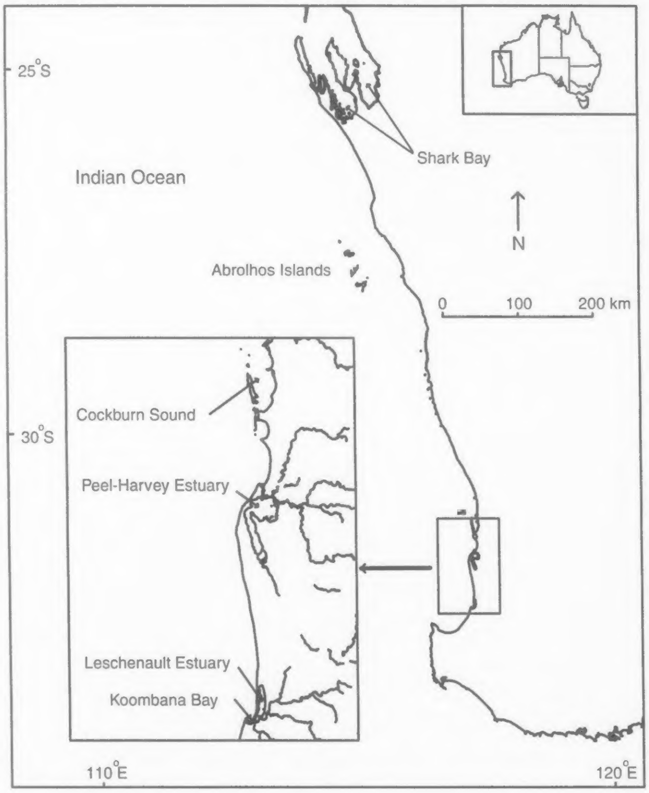


Figure 1

Map showing locations of the five bodies of water in which *Portunus pelagicus* was sampled on the west coast of Australia. The map of Australia (insert top right) shows the location (box) of the five bodies of water, and the map of the lower west of Australia (insert bottom left) shows the location of the four bodies of water sampled in this region.

Materials and methods

Sampling regimes

Up to 100 *Portunus pelagicus* were collected monthly for two years from the Leschenault Estuary (May 1997–April 1999), Koombana Bay, and Cockburn Sound (June 1998–May 2000), for three years from the Peel-Harvey Estuary (May 1995–April 1998), and bimonthly for two years from Shark Bay (July 1998–May 2000). The first four bodies of water are located on the lower west coast of Australia, approximately 800 km to the south of Shark Bay (Fig. 1). The nearshore, shallow waters in each of these bodies of water (water depth <1.5 m) were sampled for *P. pelagicus* by using a 21.5-m seine net with a bunt of 3-mm mesh; whereas offshore deeper waters were sampled by employing a small otter trawl net with a codend of 25-mm mesh and crab traps consisting of either 12- or 76-mm mesh (see Potter and de Lestang, 2000 for further details of the nets and traps). The mean water depths at the deeper offshore sites of the above five bodies of water were 3, 9, 19, 3, and 10 m, respectively. The water temperature at the bottom of the water column at each site was recorded on each sampling occasion.

Measurements and changes at puberty

The carapace width (CW) of each crab, i.e. the distance between the tips of the two lateral spines of the carapace, was measured to the nearest 1 mm. The length and height of the propodus of the largest cheliped, the length of the merus of the second walking leg, and the length of the primary pleopod of each male crab in Cockburn Sound and Shark Bay were measured to the nearest 0.1 mm. Because the relationship between the length of the dorsal propodus of the largest cheliped and the width of the carapace showed the greatest change over the size range of male crabs, that structure was chosen for allometric analysis to determine the size at which males become morphometrically mature.

Sex of small crabs, i.e. with a CW < about 30 mm, was determined with a dissecting microscope to ascertain whether their pleopods bore setae and thus the crabs were females. At CWs > about 30 mm, the female crabs could readily be distinguished from male crabs by their possession of a far wider abdominal flap (Van Engel, 1958; Warner 1977). During the pubertal molt of female portunids, the abdominal flap changes from a triangular to oval shape and from being tightly to loosely fixed to the cephalothorax (Ryan, 1967b; Fielder and Eales, 1972; Ingles and Braum, 1980; Fisher, 1999).

The size and time of occurrence of all ovigerous females were recorded. The ovary of each crab was assigned to one of four stages by using macroscopic characters similar to those described for the development of the ovaries of *P. pelagicus* and other portunids (Ryan, 1967b; Meagher, 1971; Krol et al., 1992; Kumar et al.[2]). The assignment of these stages was augmented by examining the characteristics of a subset of 200 of these ovaries in 6-μm histological sections that had been stained with Mallory's trichrome. For 5–10 ovaries of each macroscopic stage, the diameters of 30 randomly selected oocytes that had been sectioned through the nucleus were measured to the nearest 5 µm. Two measurements (the longest diameter and shortest diameter) for each oocyte were then averaged to provide an estimate of each oocyte diameter.

Male crabs were designated as either morphometrically immature or mature by using differences in the regression equations for the relationships between the natural logarithms of the length of the dorsal propodus of their largest cheliped and carapace width in what were clearly either juvenile (small and gonadally immature) or adult crabs (large and gonadally mature). For full description of the method see Somerton (1980).

On the basis of their macroscopic appearance, the vas deferentia of each male crab were assigned to one of three stages by using criteria derived from the description of gonadal development for *P. pelagicus* by Meagher (1971) and for *P. sanguinolentus* by Ryan (1967a). Aquarium studies by Meagher (1971) showed that male crabs with gonads at stages I and II did not copulate and are thus considered immature, whereas those with gonads at stage III copulated successfully with females and thus have mature gonads.

Ovaries and vas deferentia from a wide size range of at least 20 females and 20 males, respectively, from each sampling occasion in each of the five bodies of water were weighed to the nearest 0.01 g. The mean gonad weight at a constant carapace width for each sex in each month in each water body was determined by using analysis of covariance (ANCOVA) of the natural logarithm of the gonad weight as the dependent variable, month as a fixed factor, and the natural logarithm of the carapace width as a covariate. The common constant carapace width of crabs in all bodies of water was a default value calculated by the ANCOVA.

Size frequency and reproductive data for the corresponding months in the different years in each water body were pooled for describing intra-annual trends in these variables.

Size at maturity

The percentages of female crabs of different carapace widths which, in each water body, had undergone a pubertal molt, were subjected to logistic regression to determine the size at which 50% of the female crabs would have become mature *sensu* Hartnoll (1974). Data for each assemblage were randomly resampled and analyzed to create 1000 sets of bootstrap estimates of the parameters of the logistic regression and estimates of the probability of maturity within the range of recorded carapace widths. The 95% confidence intervals of the CW_{50}'s were derived by using this resampling technique, which produced slightly more conservative estimates than those obtained from the Hessian matrix of the logistic regression and thus reflected better the uncertainty of the parameter that was associated with the data. The 95% confidence intervals of the probability of maturity at each specified carapace width were taken as the 2.5 and 97.5 percentiles of the corresponding predicted values resulting from this resampling analysis. The point estimate of each parameter and of each probability of maturity at the specified carapace width were taken as the medians of the bootstrap estimates.

The percentages of mature male crabs at different carapace widths in each of the five bodies of water, with maturity being assigned by using firstly morphometric and then gonadal criteria (see earlier), were subjected to logistic regressions to determine the CW_{50}'s for these variables. The percentages of male crabs in Cockburn Sound and Shark Bay, which possessed an abdomen that was loosely fixed to the cephalothorax, were likewise subjected to logistic regression analysis. The logistic regressions relating maturity and carapace width for both the females and males in the different assemblages were compared by using a likelihood ratio test, as described by Cerrato (1990) and employing a Bonferroni correction.

Fecundity

The total wet weight of eggs in each batch of eggs of 40 early-stage ovigerous females, i.e. with yellow eggs, from Cockburn Sound and which covered a wide size range, was weighed to the nearest 0.001 g. The number of eggs in each of four replicate subsamples from each batch were recorded, after which each of those subsamples was weighed to the nearest 0.001 g. These data were then used to estimate the total number of eggs in each batch of eggs of each female. The relationship between batch fecundity (BF) and carapace width (CW) was described by using the equation $\ln BF_j = m \ln CW_j + b$.

The number of batches of eggs produced by a full size range of mature females during the spawning period was estimated by determining the spawning period (SP), defined as the time (days) when > 5% of all mature females were ovigerous, and the proportions of ovigerous females among all mature females in sequential 10-mm CW intervals during the spawning period. The proportion of ovigerous females (O_j) in the *j*th size class during this period also represents the average time a mature female in this size class is ovigerous during that period and takes into account the fact that an ovigerous female spawns at least once during a spawning period and that the brood period (*BP*) of an ovigerous female is about 18 days at 20°C (Meagher, 1971). Thus, the mean number of batches (NB_j) produced by the mature female crabs in the *j*th size class during a spawning period (average water temperature 20.4°C) can be estimated with the equation $NB_j = O_j SP/BP$.

---

[2] Kumar, M., Y. Xiao, H. Williams, G. Ferguson, G. Hooper, and S. Venema. 1999. Blue crab fishery. South Australian Fisheries Assessment Series, 99/02, 64 p. South Australian Research Development Institute, Grenfell Centre Level 14, 25 Grenfell Street Adelaide 5000, Australia.

The relationship between number of broods and carapace width was described empirically by fitting a modified logistic curve, $NB = 1 + Nb_{max}/\{1+\exp[-\ln(19)(CW-a)/(b-a)]\}$, ranging upwards from a minimum of one batch to a maximum of $1+NB_{max}$ batches, where a and b are parameters. The total fecundity of crabs at different carapace widths was calculated as the product of batch fecundity, BF, and the number of broods, NB, by using the relationships between BF and CW and NB and CW, as described above.

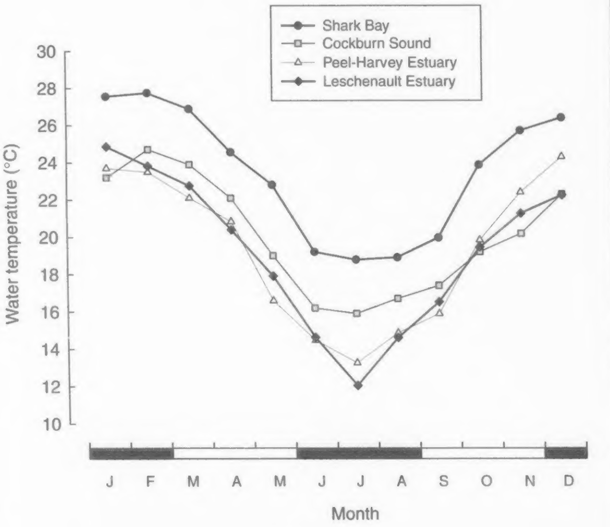


Figure 2

Mean monthly water temperatures for sampling sites in Shark Bay, Cockburn Sound, Peel-Harvey Estuary, and Leschenault Estuary (water temperatures in Koombana Bay were essentially the same as those in Leschenault Estuary). Mean monthly water temperatures in each water body were derived from data pooled for at least two years. The black rectangles on the x axis refer to summer and winter months and open rectangles to autumn and spring months.

Results

Water temperature

Mean monthly water temperatures at the bottom of the water column in the Leschenault Estuary, Peel-Harvey Estuary, Cockburn Sound, and Shark Bay followed the same trends, with values rising to a maximum in mid to late summer and declining to a minimum in mid-winter (Fig. 2). Water temperatures in Koombana Bay were essentially the same as those in Leschenault Estuary. Although the mean monthly water temperatures in the Leschenault and Peel-Harvey estuaries and Koombana Bay in corresponding months were similar, they were lower in these bodies of water than in Cockburn Sound in eight of the twelve months of the year (Fig. 2). However, the mean water temperatures in each month in Shark Bay were greater than those in the corresponding months in each of the above four more southern bodies of water. Thus, for example, although the maximum mean monthly water temperature was 28°C in Shark Bay, it never reached 25°C in any of the other bodies of water (Fig. 2). Likewise, the minimum monthly water temperature was greater in Shark Bay (19°C) than in either Cockburn Sound (16°C) or the Leschenault and Peel-Harvey estuaries (12–13°C).

Macroscopic and histological gonad staging

Macroscopic examination of the gonads of a large number of females and males of *P. pelagicus*, covering a wide size range, and, in the case of females, an histological examination of the ovaries of a subset of these crabs, showed that the ovaries and vas deferentia could be classified into four and three developmental stages, respectively (Tables 1 and 2).

Size at sexual maturity

The minimum carapace widths of female crabs that had undergone their pubertal molt ranged from 61 mm in both the Peel-Harvey Estuary and Shark Bay to 84 mm in the Leschenault Estuary. Although the CW_{50}'s derived for females at maturity in Cockburn Sound (86.4 mm) and Koombana Bay (86.9 mm) were not significantly different ($P>0.05$), both of these values were significantly less ($P<0.05$) than the 92.0 mm for females in Shark Bay (Fig. 3). The high CW_{50}'s for female crabs in the Peel-Harvey (97.5 mm) and Leschenault estuaries (98.0 mm) were not representative of females in their populations as a whole (see "Discussion" section).

The relationships between the dorsal length of the largest cheliped and carapace width of male *P. pelagicus* in each of the five bodies of water were described better by using two log-log lines (Fig. 4A) rather than a single log-log line. The CW_{50}'s of male crabs at morphometric maturity in the four bodies of water on the lower west coast, estimated with data obtained from an allometric approach and employing the above log-log regressions, ranged only from 86.2 mm in the Peel-Harvey Estuary and Cockburn

Table 1

Morphological characteristics of macroscopic stages in the development of the ovaries of *Portunus pelagicus* and the types of oocytes found in each of those stages. Mean diameters of oocytes at different stages in development are shown in parentheses.

Maturity stage		Macroscopic appearance of ovary	Types of oocytes
I	Immature	Relatively small, flattened and off white to ivory in color. Anterior region is small, and does not displace the hepatopancreas. The central "H" shaped region, located in the gastric region, is loosely joined to the dorsal surface of the spermathecae. The posterior section, located in the cardiac and intestinal regions, forms two parallel lobes.	Loosely packed oocytes, comprising oogonia (5 μm) and, to a lesser extent, chromatin nucleolar oocytes (10 μm) and perinucleolar oocytes (30 μm). These three types of oocytes are found in each of the next three ovarian stages.
II	Early development	Conspicuously larger than stage-I ovaries, pale yellow, oval in cross section and slightly nodulated. The anterior region marginally displaces the hepatopancreas and the central region envelops the dorsal surface of the spermathecae, and the two lobes of the posterior region are starting to become convoluted.	Yolk-vesicle oocytes (90 μm) are present for the first time.
III	Late development	Large, yellow, and nodulated. Anterior region displaces the hepatopancreas, and the central and posterior regions occupy almost all of the space in the gastric, posterior and intestinal cavities. Most of the spermathecae are enveloped by ovarian tissue.	Early yolk-granule oocytes (130 μm) surround small areas of early stage oocytes, and some late yolk vesicle oocytes are present.
IV	Fully mature	Very large, deep yellow to orange, and highly nodulated. Hepatopancreas is now completely displaced from its former position by the enlargement of the anterior region of the ovary. The gastric, posterior, and intestinal cavities are completely filled with the enlarged central and posterior sections of the ovary. The spermathecae are totally enveloped by the ovary.	Advanced oocytes all at the late yolk-granule stage (250 μm).

Table 2

Morphological characteristics of stages in the development of the vas deferens of *Portunus pelagicus* and the location of spermatophores in those stages.

Maturity stage		External appearance of vas deferentia	Histological appearance of the vas deferentia
I	Immature I	Vas deferentia not detectable macroscopically.	NA
II	Immature II	Anterior vas deferentia (AVD) becoming enlarged, middle and posterior vas deferentia (MVD and PVD, respectively) straight and opaque.	Spermatophores present in AVD. MVD and PVD contain no spermatophores.
III	Mature I	AVD and MVD enlarged and white and PVD enlarged and convoluted but still opaque.	Spermatophores present in AVD and MVD. PVD contains no spermatophores.

Sound to 87.2 mm in the Leschenault Estuary (Fig. 4B). The CW_{50}'s for each of these bodies of water, which were not significantly different ($P>0.05$) from each other, were significantly less at $P < 0.05$ or 0.001 than the 96.0 mm determined for male crabs in Shark Bay.

The CW_{50}'s for males at gonadal maturity in each water body, derived from the prevalence of males with mature gonads, i.e. stage III (Fig. 4C), differed by only 0.3 to 2.2 mm from those derived for males in each corresponding water body by using the prevalence of morphometrically mature males (Fig. 4B). The CW_{50}'s derived for male crabs from gonadal data in the four southern bodies of water, which ranged only from 86.5 to 88.4 mm, did not differ significantly ($P>0.05$). However, on the basis of gonadal data, each of these CW_{50}'s differed significantly at $P<0.05$, 0.01, or 0.001 from the 97.0 mm estimated for male crabs in Shark Bay (Fig. 4C). These trends were parallel to those derived from morphometric data (Fig. 4B).

The logistic curves derived from gonadal data in each of the four southern bodies of water were significantly differ-

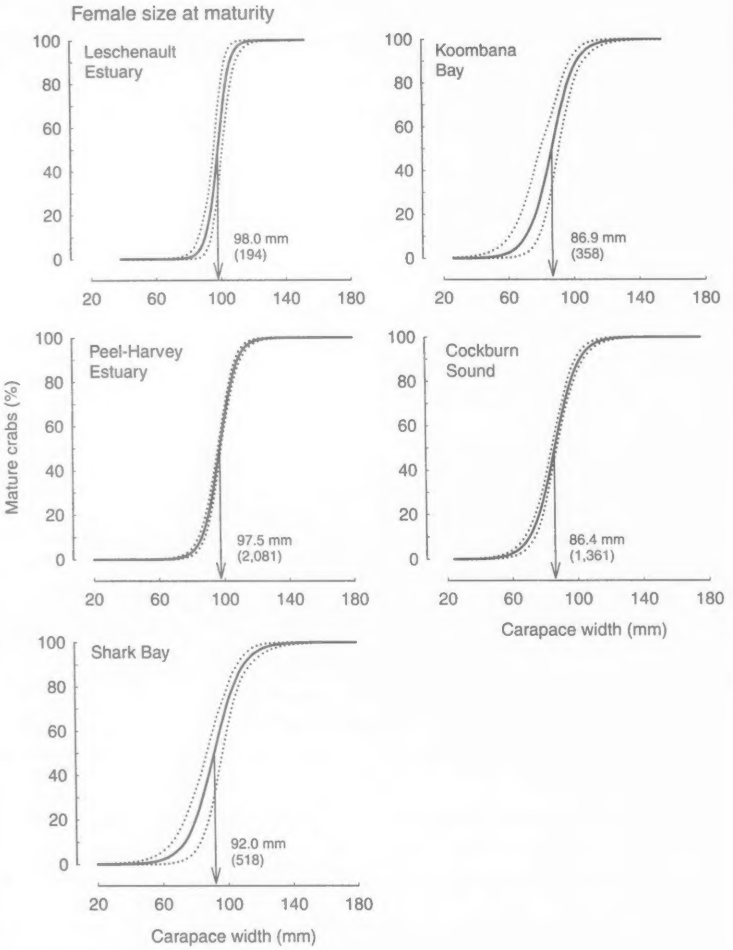


Figure 3

Logistic regressions and their 95% confidence limits fitted to percentage contributions of those adult females which, at each size, had undergone their pubertal molt in each of the five bodies of water sampled in Western Australia. Arrows and measurements denote CW_{50}'s and the numbers in parentheses refer to the number of crabs used to create the regressions.

ent ($P>0.05$) and had steeper slopes than those determined by using morphometric data (Fig. 4). The confidence limits for the logistic curves constructed from gonadal data were also usually tighter than those constructed from morphometric data.

The CW_{50} for male crabs with a loose abdominal flap in Cockburn Sound, i.e. 72.1 mm, differed significantly ($P<0.05$) from that in Shark Bay, i.e. 76.2 mm (data not shown). However, all of the male crabs in Cockburn Sound with carapace widths of 70 to 75 mm and loosely attached abdominal flaps contained gonads at stage I or II and were thus immature.

Trends exhibited by gonad weights and proportions of ovigerous females

The mean monthly gonad weight of mature female crabs with a standard carapace width (104 mm), as determined by ANCOVA (see "Material and methods" section), rose to a sharp peak of about 5 g in October in Koombana Bay and in September in Cockburn Sound (Fig. 5). In contrast, the mean monthly gonad weights of mature female crabs in the Leschenault and Peel-Harvey estuaries remained at <1.5 g and did not tend to peak sharply at any time of the year. The mean monthly gonad weights of mature female

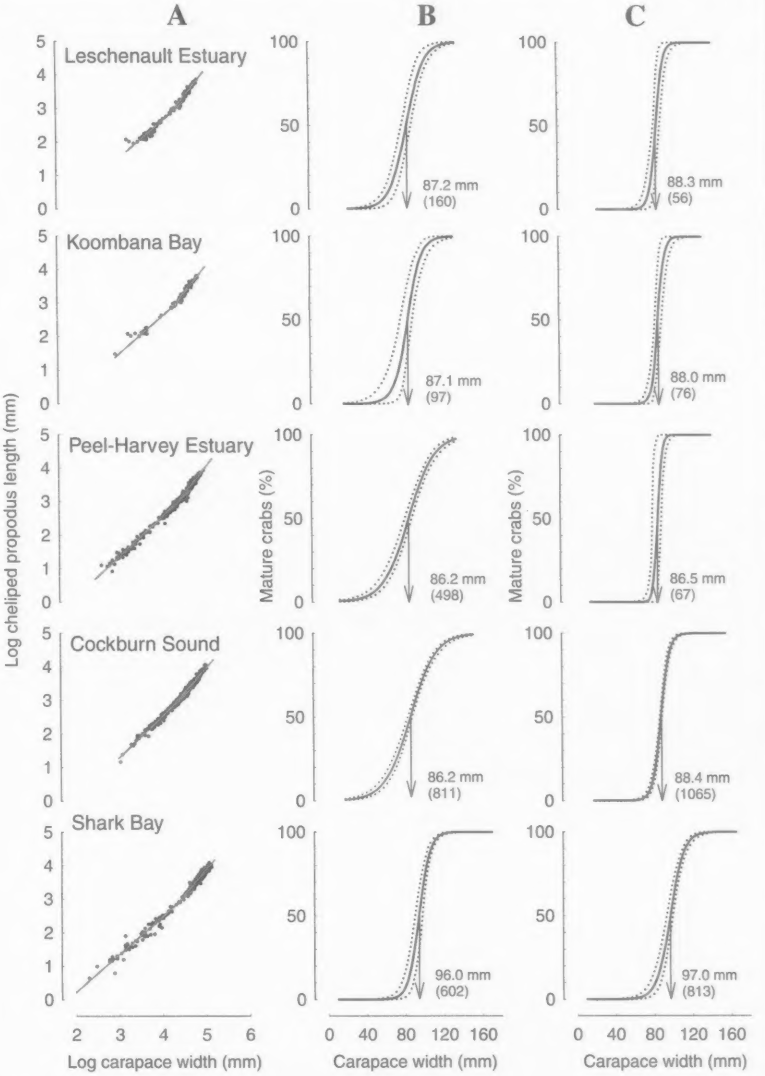


Figure 4

Maturity data for male *Portunus pelagicus* in the five bodies of water sampled in Western Australia. (**A**) Relationship between the natural log of length of the dorsal propodus of the largest cheliped and the natural log of carapace width. Logistic regressions and their 95% confidence limits were fitted to percentage contributions of those adult males, which, at each size, were (**B**) morphometrically mature and (**C**) possessed mature gonads. Arrows and measurements denote CW_{50}'s and the numbers in parentheses refer to the number of crabs used to create the regressions.

crabs in Shark Bay did not peak sharply at any time and were > 1 g in all but two of the ten months in which this embayment was sampled (Fig. 5).

The mean monthly gonad weights of male crabs with a standard carapace width of 118.4 mm, as determined by ANCOVA, varied little and never exceeded 1 g in any of the five bodies of water (data not shown). However, they did reach their maxima at a similar time of the year, i.e. late summer (February) or early autumn (March), in the four bodies of water on the lower west coast of Australia.

The monthly percentage contributions made by ovigerous female crabs from all mature female crabs in Koom-

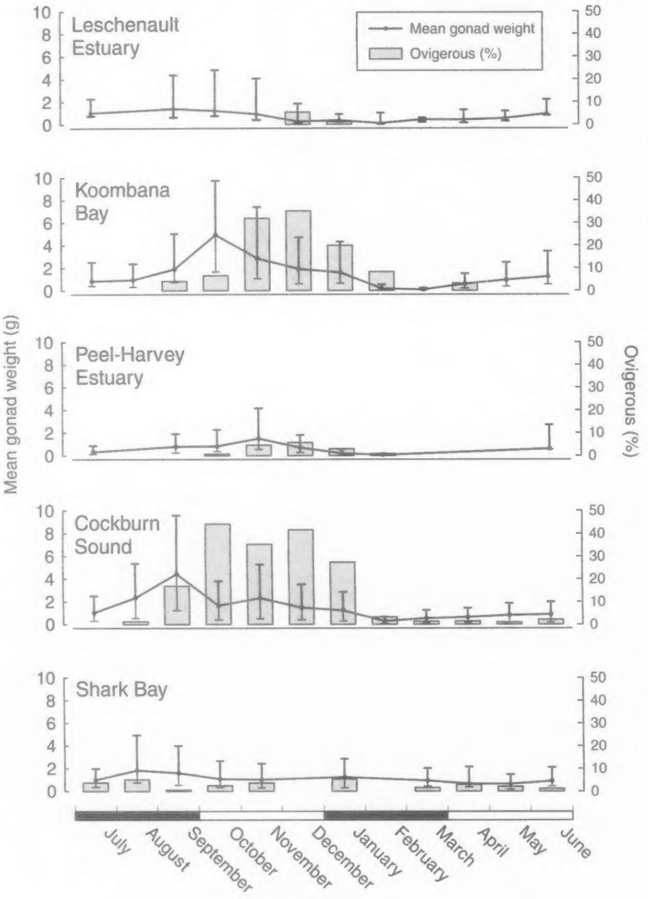


Figure 5

Mean monthly gonad weights ±95% confidence intervals for female *Portunus pelagicus* and the percentage of ovigerous females among all adult female *P. pelagicus* in the five bodies of water sampled. The black rectangles on the *x* axis refer to summer and winter months and open rectangles to autumn and spring months.

bana Bay and Cockburn Sound peaked at 32–36% in November and December in Koombana Bay and at 35–45% in October to December in Cockburn Sound. Although the corresponding percentage contributions of ovigerous female crabs were far lower in the Leschenault and Peel-Harvey estuaries than in the above two embayments, they still reached their maxima at the same time of the year (Fig. 5). Few or no ovigerous female crabs were caught in either of those estuaries or embayments between March and August. Ovigerous females were found in Shark Bay in each of the ten months in which that embayment was sampled and, unlike the situation in the two more southern marine embayments, their monthly contributions to the overall number of adult female crabs did not vary markedly throughout the year (Fig. 5).

Trends exhibited by oocyte development

The maximum diameter of the oocytes increased progressively from 95 µm in stage-I gonads to 315 µm in stage-IV gonads (data not shown). The modal oocyte diameter of the distinct and largest cohort of oocytes in stage IV (240–259 μm) was only slightly less than that of the fertilized yellow external eggs found under the abdominal flap of ovigerous females (300–319 μm). The most advanced oocytes in ovaries at stages III and IV were at the early and late yolk-granule stage, respectively. The presence of two distinct size cohorts of oocytes in the ovaries of large females with grey eggs under their abdomen (i.e. eggs that had been fertilized for several days) is consistent with the view that large female *P. pelagicus* are multiple spawners.

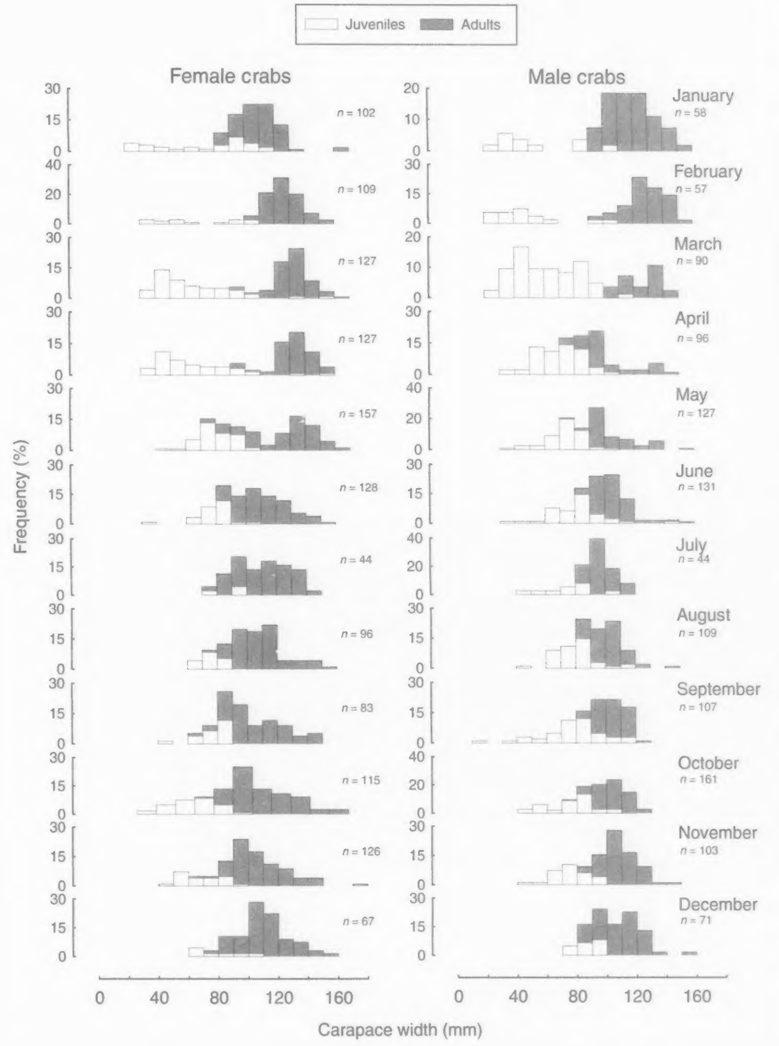


Figure 6

Frequency histograms for the carapace widths of juvenile and adult females and males of *Portunus pelagicus* in Cockburn Sound. *n* = number of crabs measured. Designation of males as mature was based on gonadal criteria described in the text.

Age and time of sexual maturation of *Portunus pelagicus*

The carapace-width frequency data for *P. pelagicus* in inshore and offshore waters in Cockburn Sound demonstrated that, in this marine embayment, males are represented by two main size cohorts in January and February (Fig. 6). The first size cohort represents the 0+ age class that resulted from the spawning period that commenced in the previous August–September, whereas the second cohort corresponds to 1+ crabs, which start to decline markedly in numbers after February and are rarely represented after June (Fig. 6). Although similar trends are exhibited by the data for females, the numbers of 1+ individuals of this sex remained higher for a longer period, i.e. until May. The above trends are entirely consistent with those reported in detailed studies of the age composition and growth of *P. pelagicus* in the Peel-Harvey Estuary (Potter et al., 1983) and Leschenault Estuary (Potter and de Lestang, 2000).

None or very few of the female and male 0+ crabs caught in Cockburn Sound in January, February, and March were mature. However, some of the larger 0+ crabs had become mature by May, i.e. when they would mostly have been

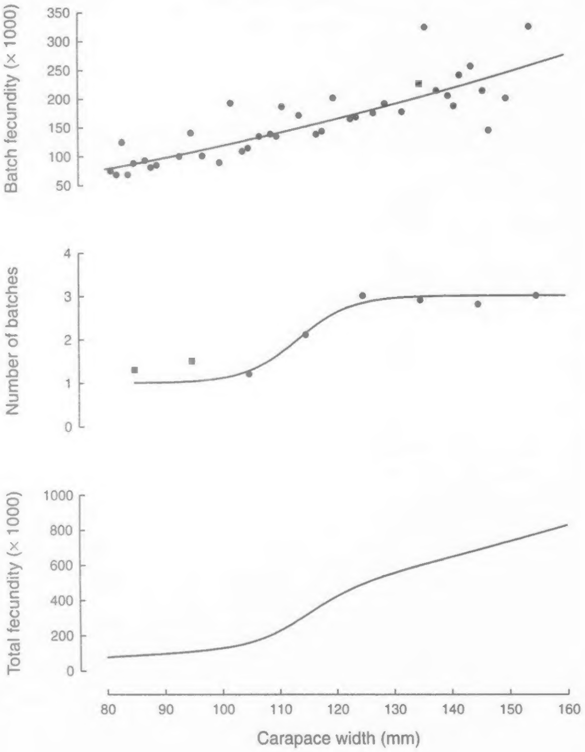


Figure 7

Batch fecundity, mean number of batches, and total fecundity of *Portunus pelagicus* in Cockburn Sound.

between four and eight months old (Fig. 6). The prevalence of mature crabs subsequently increased, with the result that the vast majority of crabs in the following January, i.e when they had just entered their second year of life, were mature (Fig. 6). Thus, all crabs have typically become mature when they are just over one year old.

Fecundity

In Cockburn Sound, the number of eggs recorded for a single batch of eggs under the abdomen of a female, ranged from 68,450 in a crab with a CW of 84 mm to 324,440 in a crab with a CW of 154 mm (Fig. 7). The relationship between batch fecundity (*BF*) and carapace width (*CW*) is described by the following equation: $\ln BF = 1.8208 \ln CW + 3.2862$.

The estimated mean number of egg batches, produced by female crabs in the different size classes over the spawning period, ranged from about one in crabs of 100–109 mm CW to about three in crabs of 150–159 mm CW (Fig. 7). A range of one to three batches per instar corresponds to that recorded by Campbell (1984) for *P. pelagicus* in aquaria experiments. The empirical relationship between the number of batches (NB) and carapace width (CW) is described by $NB_j = 1+2/\{1+\exp[-\ln(19)(CW_j-113.7)/13.8]\}$.

A combination of the equations for the relationships between batch fecundity and CW and the number of egg batches and CW was then used to determine the relationship shown between total fecundity (TF) and carapace width (CW) and which is shown in Figure 7.

Discussion

Designation of maturity in male crabs

Aquaria studies by Meagher (1971) demonstrated that male crabs with gonads at stage III, i.e. with spermatophores and seminal fluid in the medial vas deferentia, can copulate successfully with females. Because this parallels the situation recorded by Comeau and Conan (1992) for the snow crab (*Chionoecetes opilio*), we likewise regard such gonads as mature. Our study also showed that, because male *P. pelagicus* still possess immature gonads (stage II) when their abdominal flap becomes loosely attached to the cephalothorax, the latter change occurs at a prepubertal molt and thus, unlike the supposition of Sumpton et al. (1994), does not coincide with the attainment of maturity. The situation in males thus contrasts with that in female *P. pelagicus*, in which the abdominal flap becomes loose as an outcome of the pubertal molt (Fielder and Eales, 1972; Campbell, 1984; Potter and de Lestang, 2000; Smith[3]).

The very close similarity between the corresponding CW_{50}'s derived for male *P. pelagicus* in each of the five bodies of water by using morphometric and gonadal data demonstrates that morphological and gonadal maturity are attained by this species at essentially the same carapace width. However, the question of whether a male crab of about the size of maturity has become morphometrically mature depends on determining whether the relative length of one of its appendages is closer to the regression line which relates the length of that appendage to the carapace width in either juvenile or adult crabs. Because the overall relationship between cheliped length and carapace width of *P. pelagicus* does not undergo a marked shift at around the attainment of maturity, the use of the allometric method never enabled us to determine with absolute certainty whether, in the region of size overlap, a male was morphometrically immature or mature. The lack of precision, when determining maturity with morphometric data, could account for the slopes of the logistic curves for the prevalence of "mature" individuals of *P. pelagicus* derived from these morphometric data in the four southern bodies of water being shallower than those obtained from gonadal data.

From the above, it follows that there would be an advantage in determining the CW_{50}'s for male *P. pelagicus* at maturity by using data on gonadal state obtained by the simple and direct procedure of examining the vas deferentia, rather than relying on data obtained by an allometric method that is indirect and relies on a careful measurement of the appendage lengths and carapace dimensions of a considerable number of individuals. However, the remarkable similarities between the CW_{50}'s derived by using morphometric and gonadal data show that, if it is desirable to avoid damaging the crabs, the data obtained from allometric analysis does yield a close approximation of this important measure for *P. pelagicus*. Thus, the CW_{50} derived from either gonadal or morphometric data for male *P. pelagicus* can be used for developing management plans for this species.

The very close correspondence between the size at which gonadal and morphometric maturity are attained by the males of *P. pelagicus* contrasts with the situation recorded by Comeau and Conan (1992) and Sainte-Marie et al. (1997) for the males of the snow crab *Chionoecetes opilio*. In this latter species, the males attain gonadal maturity at a smaller body size than that at which morphometric maturity is attained following the terminal molt. The males of *C. opilio* with large cheliped and large body size are at a competitive advantage over smaller males when courting (Comeau and Conan, 1992; Sainte-Marie et al., 1997). Because the aquaria studies of Campbell (1984) have shown that the large males of *P. pelagicus* also have a similar competitive advantage during courting, a male of this species with mature gonads and differentiated chelipeds may not be able to compete for females successfully if larger males are present.

Influence of migration on estimates of CW_{50} for female crabs

The mean monthly gonad weights recorded for post-pubertal individuals were less for females in estuaries than in marine embayments, strongly indicating that females often tend to emigrate from the estuaries to their spawning grounds before their gonads are fully developed (Van Engel, 1958; Potter and de Lestang, 2000). Such an emigration from estuaries by mature female *P. pelagicus* reduces the proportion of mature individuals within each carapace width interval, thereby increasing the proportion of immature females in these class intervals. This shifts the logistic curve to the right and consequently increases the CW_{50}, which accounts for the significantly greater CW_{50}'s derived for females in estuaries than in marine environments on the lower west coast of Australia. For this reason, subsequent comparisons of the CW_{50}'s for female crabs in the different bodies of water will focus on those derived for assemblages in the three marine embayments.

In contrast to the CW_{50}'s for females, the CW_{50}'s for males at maturity in the two estuaries and the two marine embayments on the lower west coast of Australia were not significantly different. This presumably reflects the fact that, unlike mature females, the large males of *P. pelagicus* tend to remain in estuaries during the spawning period (Potter and de Lestang, 2000).

Influence of temperature on reproductive biology

The CW_{50}'s derived for males at "maturity" in each of the five bodies of water never differed by more than 2.2 mm, irrespective of whether gonadal or morphometric data were used. However, the maximum CW_{50}'s determined for males in the two estuaries and two embayments by using gonadal and morphometric data, i.e. 88.4 mm for Cockburn Sound and 87.2 mm for the Leschenault Estuary, respectively, were 8.6 and 8.8 mm less than the corresponding CW_{50}'s determined for males in Shark Bay. Furthermore, the CW_{50}'s for female *P. pelagicus* in Koombana Bay and Cockburn Sound were 5.6 and 5.1 mm, respectively, less than that of females in Shark Bay.

The greater CW_{50}'s for *P. pelagicus* in Shark Bay than in the other four bodies of water, which are located approximately 800 km farther south, runs counter to the generalization that the CW_{50}'s for decapods tend to be inversely related to water temperature (e.g. Campbell and Robinson, 1983; Jones and Simons, 1983; Dugan et al., 1991). However, the opposite situation has sometimes been recorded and, in those cases, has been attributed to differences among populations of one or more of the following: density, predation pressure, and food availability (Hines, 1989; Polovina, 1989; Pollock, 1995; McGarvey et al., 1999). It thus appears relevant that the mean density of *P. pelagicus* was far lower in the sampling sites in Shark Bay, 0.6 crabs/100 m^2, than those in Cockburn Sound and Koombana Bay, 2.80 and 2.94 crabs/100 m^2, respectively. The mean density in Shark Bay was also far lower than those recorded in the Leschenault and Peel-Harvey estuaries between the middle of spring and middle of autumn, when *P. pelagicus* colonizes estuaries (Potter et al., 1983; Potter and de Lestang, 2000). Furthermore, commercial or recreational fishing pressure (or both), which leads to a reduction in CW_{50}'s at maturity in the spiny lobster (Polovina, 1989), is far greater for *P. pelagicus* in the southern bodies of water than in Shark Bay (Bellchambers[4]). Recent work with microsatellite DNA has also shown that the assemblages of *P. pelagicus* in Shark Bay are genetically distinct from those in more southern bodies of water, such as Cockburn Sound and the Peel-Harvey Estuary (Chaplin et al.[5]).

---

[3] Smith, H. 1982. Blue crabs in South Australia—their status, potential and biology. Report 6, p. 33–51. South Australian Fisheries Industry Council Grenfell Centre Level 14, 25 Grenfell Street Adelaide 5000, Adelaide, Australia.

The marked differences between the CW_{50}'s at maturity for *P. pelagicus* in Shark Bay and bodies of water farther south emphasize the need for managers to take into account this type of variation when determining a minimum legal carapace width (MLCW) for capture. However, the current MLCW for *P. pelagicus* in Western Australia, 127 mm, is well above even the CW_{50} for this species at maturity in Shark Bay.

The prevalence of ovigerous females did not peak sharply at any time of the year in Shark Bay, whereas ovigerous females were found predominantly during spring and summer in Cockburn Sound and Koombana Bay. Moreover, the mean monthly gonad weights of a female *P. pelagicus* of standard carapace width lay within a relatively narrow range of 0.9 to 1.8 g in Shark Bay, whereas they rose to a sharp peak of about 5 g in spring and fell below 1 g in some months in Cockburn Sound and Koombana Bay. The trends exhibited by the reproductive variables of female *P. pelagicus* thus provided strong evidence that reproductive activity extends over much or all of the year in Shark Bay, whereas it occurs predominantly in spring and summer in the two southern embayments. The more protracted spawning period in Shark Bay presumably reflects the presence of higher water temperatures throughout the year and in particular during winter and early spring. Such a conclusion is consistent with the results of other studies, which have shown that water temperature influences ovulation and egg development in *P. pelagicus* and other decapods (Rahaman, 1980; Campbell, 1984; Pollock, 1995; Kumar et al.[2]).

Fecundity

The vast majority of previous estimates of the fecundity of crustaceans have been based on the number of eggs borne by females at a particular time which, in the case of multiple spawners, does not take into account the fact that larger crabs can produce two or more batches of eggs within a spawning period. The few previous attempts to obtain the total fecundity of crustaceans have involved tracking the number of batches of eggs borne by particular individuals at different times (e.g. Chubb et al.[6]). The advantage of the approach developed during the current study is that it uses a combination of batch fecundity and an estimate of the number of batches produced during the spawning period by female *P. pelagicus* of different carapace widths to determine the relationship between the total fecundity and body size of this species in a given population. Because the older crabs have a far longer intermolt period between copulation and egg extrusion than younger crabs, i.e. eight versus four months, they have a far greater amount of time to accumulate the energy reserves required to produce eggs. This difference accounts for the greater number of egg batches produced by larger than small crabs.

Acknowledgments

Thanks are expressed to many colleagues and friends, and particularly R. Melville-Smith, D. Fairclough, M. Pember, T. Linke, M. Travers, and W. White, who assisted with sampling. Our thanks are also expressed to the three anonymous referees for their constructive criticisms. Funding was provided by the Australian Fisheries Research and Development Corporation and Murdoch University.

Literature cited

Anonymous.
2000. FAO yearbook. Fisheries statistics. Capture production, 86/1, 713 p. FAO, Rome, Italy.

Campbell, G. R.
1984. A comparative study of adult sexual behaviour and larval ecology of three commercially important portunid crabs from the Moreton Bay region of Queensland, Australia. Ph.D. diss. 253 p. Univ. Queensland, Brisbane, Queensland, Australia.

Campbell, A., and D. G. Robinson.
1983. Reproductive potential of three American lobster (*Homarus americanus*) stocks in the Canadian Maritimes. Can. J. Fish. Aquat. Sci. 40:1958–1967.

Cerrato, R. M.
1990. Interpretable statistical tests for growth comparisons using parameters in the von Bertalanffy equation. Can. J. Fish. Aquat. Sci. 47:1416–1426.

Comeau, M., and G. Y. Conan.
1992. Morphometry and gonad maturity of male snow crab, *Chionoecetes opilio*. Can. J. Fish. Aquat. Sci. 49: 2460–2468.

Dugan, J. E., A. M. Wenner, and D. M. Hubbard.
1991. Geographic variation in the reproductive biology of the sand crab *Emerita analoga* (Stimpson) on the California coast. J. Exp. Mar. Biol. Ecol. 150:63–81.

---

[4] Bellchambers, L. 2002. Personal commun. Fisheries Western Australia, WA Marine Research Laboratories, West Coast Drive, Waterman, 6020, Perth, Australia.

[5] Chaplin, J., E. S. Yap, E. Sezmis, and I. C. Potter. 2001. Genetic (microsatellite) determination of the stock structure of the blue swimmer crab in Australia. FRDC project 98/118, 84 p. Murdoch University, South Street, Murdoch, 6150, Perth, Australia.

[6] Chubb, C., C. Dibden, and K. Ellard. 1984. Studies on the breeding stock of the western rock lobster, *Panulirus cygnus*, in relation to stock and recruitment. FIRTA project 85/87, 37 p. Fisheries Western Australia, WA Marine Research Laboratories, West Coast Drive, Waterman, 6020, Perth, Australia.

Fielder, D. R., and A. J. Eales.
1972. Observations on courtship, mating and sexual maturity in *Portunus pelagicus* (L., 1766). J. Nat. Hist. 6: 273–277.

Fisher, M. R.
1999. Effect of temperature and salinity on size at maturity of female blue crabs. Trans. Am. Fish. Soc. 128:499–506.

Hartnoll, R. G.
1974. Variation in growth pattern between some secondary sexual characters in crabs (Decapoda, Brachyura). Crustaceana 27:131–136.

Hill, B. J.
1975. Abundance, breeding and growth of the crab *Scylla serrata* in two South African estuaries. Mar. Biol. 32: 119–126.

Hines, A. H.
1989. Geographic variation in size at maturity in brachyuran crabs. Bull. Mar. Sci. 45:356–368.

Ingles, J., and E. Braum.
1989. Reproduction and larval ecology of the blue swimming crab *Portunus pelagicus* in Ragay Gulf, Philippines. Int. Rev. Hydrobiol. 74:471–490.

Jones, M. B., and M. J. Simons.
1983. Latitudinal variation in reproductive characteristics of a mud crab, *Helice crassa* (Grapsidae). Bull. Mar. Sci. 33:656–670.

Kailola, P. J., M. J. Williams, P. C. Stewart, R. E. Riechelt, A. McNee, and C. Grieve (eds.).
1993. Australian fisheries resources, 422 p. Bureau of Resource Sciences, Canberra, Australia.

Krol, R. M., W. E. Hawkins, and R. M. Overstreet.
1992. Reproductive components. *In* Microscopic anatomy of invertebrates (F. W. Harrison and A. G. Humes, eds.), p. 295–343. Wiley-Liss, New York, NY.

McGarvey, R., G. J. Ferguson, and J. H. Prescott.
1999. Spatial variation in mean growth rates at size of southern rock lobster, *Jasus edwardsii*, in South Australian waters. Mar. Freshw. Res. 50:333–342.

Meagher, T. D.
1971. Ecology of the crab *Portunus pelagicus* (Crustacea: Portunidae) in south Western Australia. Ph.D. diss, 227 p. Univ Western Australia, Perth, Australia.

Melville-Smith, R.
1987. The reproductive biology of *Geryon maritae* (Decapoda, Brachyura) off south west Africa/Namibia. Crustaceana 53:259–275.

Metcalf, K. S., J. van Montfrans, R. N. Lipcius, and R. J. Orth.
1995. Settlement indices for blue crab megalopae in the York River, Virginia: temporal relationships and statistical efficiency. Bull. Mar. Sci. 57:781–792.

Miliou, H.
1996. The effect of temperature, salinity and diet on final size of female *Tisbe holothuriae* (Copepoda, Harpacticoida). Crustaceana 69:742–754.

Muiño, R., L. Fernandez, E. Gonzalez-Gurriaran, J. Freire, and J. A. Vilar.
1999. Size at maturity of *Liocarcinus depurator* (Brachyura: Portunidae): a reproductive and morphometric study. J. Mar. Biol. Assoc. U.K. 79:295–303.

Perkins-Visser, E., T. G. Wolcott, and D. L. Wolcott.
1996. Nursery role of seagrass beds: enhanced growth of juvenile blue crabs (*Callinectes sapidus* Rathburn). J. Exp. Mar. Biol. Ecol. 198:155–171.

Pillai, K. K., and N. B. Nair.
1971. The annual reproductive cycles of *Uca annulipes*, *Portunus pelagicus* and *Metapenaeus affinis* (Decapoda: Crustacea) from the south-west coast of India. Mar. Biol. 11:152–166.

Pollock, D. E.
1995. Changes in maturation ages and sizes in crustacean and fish populations. S. Afr. J. Mar. Sci. 15:99–103.

Polovina, J. J.
1989. Density dependence in spiny lobster, *Panulirus marginatus*, in the northwestern Hawaiian Islands. Can. J. Fish. Aquat. Sci. 46:660–665.

Potter, I. C., P. J. Chrystal, and N. R. Loneragan.
1983. The biology of the blue manna crab *Portunus pelagicus* in an Australian estuary. Mar. Biol. 78:75–85.

Potter, I. C., and S. de Lestang.
2000. Blue swimmer crab *Portunus pelagicus* in Leschenault Estuary and Koombana Bay, south-western Australia. J. R. Soc. West. Aust. 83:221–236.

Prager, M. H.
1996. A simple model of the blue crab, *Callinectes sapidus*, spawning migration in Chesapeake Bay. Bull. Mar. Sci. 58:421–428.

Rahaman, A. A.
1980. Ecological observations on spawning of a few invertebrates of the Madras coast. J. Madurai Kamaraj Univ. 9:71–77.

Reeby, J., P. N. Prasad, and M. S. Kusuma.
1990. Size at sexual maturity in the male crabs of *Portunus sanguinolentus* and *P. pelagicus*. Fish. Technol. 27: 115–119.

Ryan, E. P.
1967a. Structure and function of the reproductive system of the crab *Portunus sanguinolentus* (Herbst) (Brachyura: Portunidae). I. The male system. Mar. Biol. Assoc. India Symp. Ser. 2:506–521.
1967b. Structure and function of the reproductive system of the crab *Portunus sanguinolentus* (Herbst) (Brachyura: Portunidae). II. The female system. Mar. Biol. Assoc. India Symp. Ser. 2:522–544.

Sainte-Marie, B., J. M. Sévigny, and Y. Gauthier.
1997. Laboratory behaviour of adolescent and adult males of the snow crab (*Chionoecetes opilio*) (Brachyura: Majidae) mated noncompetitively and competitively with primiparous females. Can. J. Fish. Aquat. Sci. 54:239–248.

Somerton, D. A.
1980. A computer technique for estimating the size of sexual maturity in crabs. Can. J. Fish. Aquat. Sci. 37: 1488–1494.

Somerton, D. A., and W. Donaldson.
1996. Contribution to the biology of the grooved and triangle tanner crabs, *Chionoecetes tanneri* and *C. angulatus*, in the eastern Bering Sea. Fish. Bull. 94:348–357.

Stephenson, W.
1962. The evolution and ecology of portunid crabs, with especial reference to Australian species. *In* The evolution of living organisms (G. W. Leeper, ed.), p. 34–67. Melbourne Univ. Press, Melbourne, Australia.

Sumpton, W. D., M. A. Potter, and G. S. Smith.
1994. Reproduction and growth of the commercial sand crab, *Portunus pelagicus* (L.) in Moreton Bay, Queensland. Asian Fish. Sci. 7:103–113.

Van Engel, W. A.
1958. The blue crab and its fishery in Chesapeake Bay. Part 1. Reproduction, early development, growth and migration. Commer. Fish. Rev. 20:6–17.

Warner, G. F.
1977. The biology of crabs, 202 p. Paul Elek (Scientific Books), London.

A model for assessing the likelihood of self-sustaining populations resulting from commercial production of triploid Suminoe oysters (*Crassostrea ariakensis*) in Chesapeake Bay

Jodi R. Dew
Jim Berkson
Eric M. Hallerman
Department of Fisheries and Wildlife Sciences
106 Cheatham Hall
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061-0321
E-mail address (for J. Berkson, contact author): jberkson@vt.edu

Standish K. Allen Jr.
School of Marine Science
Virginia Institute of Marine Sciences
Gloucester Point, Virginia 23062

Abstract—Culture of a non-native species, such as the Suminoe oyster (*Crassostrea ariakensis*), could offset the harvest of the declining native eastern oyster (*Crassostrea virginica*) fishery in Chesapeake Bay. Because of possible ecological impacts from introducing a fertile non-native species, introduction of sterile triploid oysters has been proposed. However, recent data show that a small percentage of triploid individuals progressively revert toward diploidy, introducing the possibility that Suminoe oysters might establish self-sustaining populations. To assess the risk of Suminoe oyster populations becoming established in Chesapeake Bay, a demographic population model was developed. Parameters modeled were salinity, stocking density, reversion rate, reproductive potential, natural and harvest-induced mortality, growth rates, and effects of various management strategies, including harvest strategies. The probability of a Suminoe oyster population becoming self-sustaining decreased in the model when oysters are grown at low salinity sites, certainty of harvest is high, minimum shell length-at-harvest is small, and stocking density is low. From the results of the model, we suggest adopting the proposed management strategies shown by the model to decrease the probability of a Suminoe oyster population becoming self-sustaining. Policy makers and fishery managers can use the model to predict potential outcomes of policy decisions, supporting the ability to make science-based policy decisions about the proposed introduction of triploid Suminoe oysters into the Chesapeake Bay.

Manuscript approved for publication 16 June 2003 by Scientific Editor.
Manuscript received 26 June 2003 at NMFS Scientific Publications Office.
Fish. Bull. 101:758–768 (2003).

The native eastern oyster (*Crassostrea virginica*) population in Chesapeake Bay has declined because of habitat degradation, over-harvest, and disease- and parasite-mediated mortality. Efforts to restore the eastern oyster population in Maryland and Virginia have been hindered by persistent diseases and habitat degradation (Mann et al., 1991; Gottlieb and Schweighofer, 1996). Recent restoration efforts have included intensified reef building programs. In addition to restoring the native oyster, discussions about introducing non-native disease- and parasite-resistant oyster species into the Chesapeake Bay have gone forward since the early 1990s (Mann et al., 1991; Lipton et al., 1992; Gottlieb and Schweighofer, 1996; Hallerman et al., 2002).

In 1997, in-water testing of non-native oyster species (sterile triploids) began in Virginia, first with the Pacific oyster (*Crassostrea gigas*), then with the Suminoe oyster (*Crassostrea ariakensis*) (Calvo et al., 1999; Calvo et al., 2001). Field studies with Pacific oysters showed poor performance under Chesapeake Bay conditions (Calvo et al., 1999). However, field studies with Suminoe oysters demonstrated disease resistance and rapid growth, and individuals reached minimum harvest shell length of about 77 mm in approximately one year (Calvo et al., 2001). These results, and subsequent small-scale trials by industry, evoked strong interest in the commercial culture of Suminoe oysters to supplement the eastern oyster fishery.

Ideally, aquaculture with 100% triploid oysters would pose no risk of establishment of a self-sustaining oyster population (Guo and Allen, 1994a). However, a number of factors make the use of triploids imperfect. For example, recent data have shown that a small percentage of triploid oysters progressively revert toward diploidy with age (Calvo et al., 2001; Zhou, 2002). Reversion of triploids leads to mosaicism in which individuals comprise both diploid and triploid cells. Mosaics themselves are innocuous unless the re-establishment of diploid cells leads to recovered reproductive capability, which could in turn lead to the establishment of a self-sustaining Suminoe oyster population. We define this hazard, "reproductively effective reversion," as the process of yielding mosaics with recovered reproductive capability. Reproductively effective reversion introduces the possibility that triploid Suminoe oysters planted for aquaculture could become a self-sustaining population of diploid Suminoe oysters and introduce numerous unknown ecological consequences.

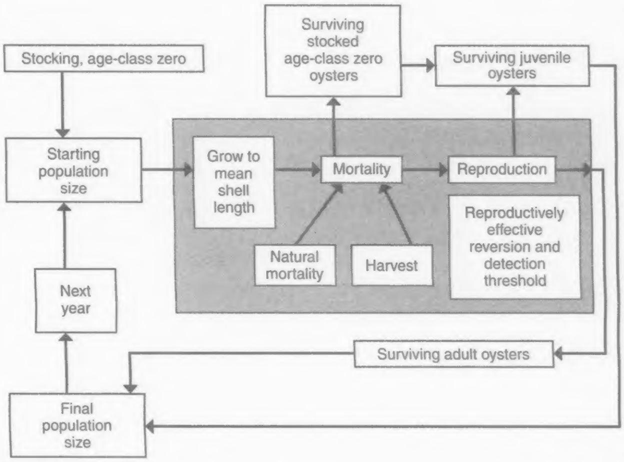

Stocking, age-class zero
Surviving stocked age-class zero oysters
Surviving juvenile oysters
Starting population size
Grow to mean shell length
Mortality
Reproduction
Reproductively effective reversion and detection threshold
Natural mortality
Harvest
Next year
Surviving adult oysters
Final population size

Figure 1

Flow chart depiction of the annual time step in the model for estimating likelihood of establishing self-sustaining reproduction in triploid Suminoe oysters (*C. ariakensis*).

Another hazard associated with deployment of triploid Suminoe oysters is the possibility that nontriploids might be stocked inadvertently because of failure to detect them in a mixed batch of triploid and diploid individuals. Although technology to produce "100%" triploids is now available, as practiced on Pacific oyster (Guo and Allen, 1994b; Guo et al., 1996), the reliability of the approach for producing "100%" triploids in Suminoe oyster is yet undetermined. Diploids may enter the population from several sources: chromosomal nondisjunction in tetraploid males producing haploid gametes, low level hermaphrodism in diploid females yielding self-fertilized embryos, and cross-contamination between diploid and triploid cultures (cf. Guo and Allen, 1997). Typically, flow cytometry has been used to determine the presence or absence of diploid cells (Allen, 1983). Flow cytometry has the sensitivity to detect one diploid among a thousand triploid oysters (Allen and Bushek, 1992); thus, the detection threshold is 0.001 with current technology. Should the (nonzero) frequency of diploids be greater than zero but less than one in a thousand, then the batch would be certified 100% triploid. This failure to detect diploid individuals in a mixed batch poses a hazard for stocking other fertile diploid oysters in that batch into culture systems.

Before substantial commercial introduction of triploid Suminoe oysters into the Chesapeake Bay, any environmental hazards of reproduction associated with a range of management scenarios should be assessed. Hazards are defined as undesirable outcomes from an activity (Hallerman and Kapuscinski, 1995). Stocking triploid Suminoe oysters produces two hazards in this model: the inadvertent stocking of diploids and the reproductively effective reversion of triploids. These two hazards may lead to the establishment of a self-sustained Suminoe oyster population and the probability of this occurring is defined as a risk. Risk assessment is the process of 1) identifying hazards posed by management actions, such as deployment of triploid Suminoe oysters, 2) quantifying the associated risks of hazards being realized (Hallerman and Kapuscinski, 1995), such as the population becoming self-sustaining, and 3) evaluating the consequences of the hazards. Quantitative models often are used to assess risk (Lackey, 1994). Building upon data collected on growth, mortality, and reproductively effective reversion for Suminoe oysters, we have developed a quantitative model to estimate the risk associated with large-scale deployment of triploid Suminoe oysters under a range of management scenarios. The model predicts the likelihood of out-planted triploid Suminoe oysters giving rise to a self-sustaining population at a given site in the Chesapeake Bay given user-specified stocking, reproductively effective reversion, reproduction, growth, and mortality rates (both natural and harvest), as well as user-specified management options.

Methods

Overview of model

A quantitative population model of the Suminoe oyster was developed to evaluate the consequences of hazards associated with introducing triploid Suminoe oysters under a range of environmental conditions and management strategies. The model includes set demographic parameters (length-fecundity, oyster density-fertilization efficiency, and salinity-fecundity relationships) and user-specified variables (reproduction, growth, and natural and harvest mortality rates). It includes options for varying stocking rates, harvest rates, and other management actions. Because little is known about Suminoe oyster reproduction, we assumed that Suminoe oysters would behave like the congeneric eastern oysters in Chesapeake Bay; hence, an eastern oyster fecundity model (Mann and Evans, 1998) was used to estimate fecundity of Suminoe oysters. The model assumes that the Suminoe oyster population is closed, i.e. that natural immigration and emigration do not occur. The model is age-structured, and a yearly time step is used. The state variable tracked through time is population size. Intrinsic population growth rate is exponential and without density dependence. The final output of the model is the predicted population size of Suminoe oyster assuming specified demographic parameters and environmental and management variables. The model was programmed in Visual Basic (Microsoft Corp., Redmond, WA).

Modeling approach

In each annual time step for age classes one through six, growth occurs to the mean shell length of the age class, then natural mortality and harvest are imposed, and then reproduction occurs (Fig. 1). Because Suminoe oysters grow quickly in autumn (Cahn, 1950), the annual time step begins in September. Harvest occurs from October to April. Natural mortality occurs at the greatest rates during the summer months. Because an annual time step is being used, the model is designed so that natural mortality and harvest are imposed simultaneously. Reproduction occurs during the summer months. The model simulates reproduction for fertile individuals in all mature age classes. The final population size for a particular age class after natural mortality and harvest becomes the starting value for population size for the next age class in the next time step. All individuals stocked each year are age-class zero individuals. The starting population size for age-class one in the next time step is equal to the sum of all individuals less than one-year old produced by all age classes, plus the number of individuals stocked.

Model variables, parameters, and equations

The initial conditions for the model are determined by the user's choice of specific values for several variables (Tables 1 and 2). The key abiotic variable driving population growth is salinity, because fecundity is highly dependent upon salinity (Mann and Evans, 1998). Biotic variables of the model include mean shell length for each age class, mortality (natural and harvest) for each age class, disease prevalence, total mortality of oysters less than one year old, oyster population density, sex ratio for each age class, and reproductively effective reversion rate for each age class (Table 1). Other variable inputs are stocking rates, harvest regulations, and management strategies.

Stochasticity is programed into the model to incorporate both the uncertainty involved in estimating variable values and environmental variation. Some variables are regarded as stochastic variables because they vary around some mean value from year to year, whereas other variables (such as salinity and sex ratio of the population for each age class) are deterministic in the model because they fluctuate over a longer period of time in the absence of a catastrophe (Kennedy et al., 1996). Stochasticity affects shell length, natural mortality, and reproductively effective reversion rates at each age, and the degree of variance is set by the user as a constant for each year. At each time step, a mean shell length, mortality rate, and reproductively effective reversion rate for each age class is randomly drawn from a log normal distribution around a mean with an associated variance.

We assume that the mean shell length of each age class at the current time step does not affect the mean length of the age class at a subsequent time step, because of large, highly variable growth rates per year (Calvo et al., 2001). Default mean shell length for each age-class values were obtained from Cahn (1950). The user may, of course, specify other mean shell lengths. Growth affects the potential for recruitment through the effect of shell length on fecundity in Equation 1 below.

Table 1

Definitions of model parameters and variables.

Symbol	Parameter and variable definition
A	Area (square meters)
C	Certainty in obtaining the desired harvest rate ($0 \le C \le 1$)
$D_{t,i}$	Oyster density (number of oysters per m^2) at time t for age-class i
$F_{t,i}$	Total fecundity (number of eggs) at time t for age-class i
Fd	Effect of disease on fecundity ($0 \le Fd \le 1$)
$Ff_{t,i}$	Fertilization efficiency at time t for age-class i ($0 \le Ff_{t,i} \le 1$)
Fq_i	Effect of sex ratio on fecundity for age-class i ($0 \le Fq_i \le 1$)
$Frevert_{t,i}$	Total fecundity (number of eggs) of reverted triploid oysters at time t for age-class i
Fs	Effect of salinity on fecundity ($0 \le Fs \le 1$)
$Ftotal_t$	Modified total number of offspring produced at time t for all age classes
H_i	Harvest mortality for age-class i
i	Age class
K_t	Number of stocked oysters at time t
$L_{t,i}$	Mean shell length (mm) at time t for age-class i
$Lmort$	Daily larval mortality rate until settlement
$M_{t,i}$	Natural mortality at time t for age-class i
$N_{t,i}$	Population size (number of oysters) at time t for age-class i
$Ntotal_t$	Total population size (number of oysters) for all age classes at time t
P_{met}	Probability of successful metamorphis ($0 \le P_{met} \le 1$)
$R_{t,i}$	Reproductively effective reversion rate at time t for age-class i ($0 \le R_{t,i} \le 1$)
$T_{t,i}$	Detection threshold at time t for age-class i ($0 \le T_{t,i} \le 1$)
S	Salinity (ppt)
t	Time (years)

An equation for individual size-specific fecundity presented by Mann and Evans (1998) was multiplied by the numbers and mean sizes of females in each mature age class in order to estimate total fecundity for a diploid population:

$$F_{t,i} = 39.06 \times [0.000423 \times L_{t,i}^{1.17475}]^{2.36} \times N_{t,i}, \quad (1)$$

where $F_{t,i}$ = total fecundity (number of eggs produced) at time t for age-class i greater than one;

Table 2

Default values and age-class default values set in the model.

Variable	Default value
A (square meters)	220
C	0.9
Extra timesteps without stocking (years)	20
Fd	1.0
H_t	1.0
Iterations	350
K_t	10000
Minimum shell length-at-harvest (mm)	76.6
S (ppt)	15
t (years)	50

	Age-class default values						
Variable	$i = 0$	$i = 1$	$i = 2$	$i = 3$	$i = 4$	$i = 5$	$i = 6$
Fq_i [1]		0.28	0.66	0.8	0.9	0.95	0.95
$L_{t,i}$ (mm) [2]		54.5	96.9	124.2	151.5	178.7	196.9
$M_{t,i}$ [3]	0.98	0.2	0.2	0.2	0.2	0.2	0.2
$R_{t,i}$ [3]		0	0	0.049	0.009	0.014	0.019
$T_{t,i}$ [3]		0.001	0.001	0.001	0.001	0.001	0.001
Variance of $L_{t,i}$ (mm) [3]		5	10	10	10	10	10
Variance of $M_{t,i}$ [3]		0.05	0.05	0.05	0.05	0.05	0.05
Variance of $R_{t,i}$ [3]		0.0005	0.0005	0.0005	0.005	0.005	0.005

[1] (Yingya et al., 1992).
[2] (Cahn, 1950).
[3] (Calvo et al., 2001).

$L_{t,i}$ = mean shell length (mm) at time *t* for age-class *i* greater than one; and
$N_{t,i}$ = population size (number of diploid oysters) at time *t* for age-class *i* greater than one.

We recognize that growth forms in oysters are fluid, and that fecundity may be more closely related to weight than to length. However, available growth data for *C. ariakensis* described length-at-age, and therefore we modified Mann and Evans' (1998) fecundity equation to use length as the independent variable.

Equation 1 is used when determining the number of eggs produced by diploid oysters that survived from the previous year. Equation 1 must be modified to determine the number of eggs produced by triploid oysters undergoing reproductively effective reversion. The reproductively effective reversion rate comprises two reproduction-related processes, nondetection of diploids ($T_{t,i}$) and reproductively effective reversion ($R_{t,i}$) of triploids. First, diploid individuals may enter the population through a failure to detect them at frequencies lower than 0.001 in mixed batches with triploids. We make the ecologically conservative assumption that diploid individuals will enter the first age class at the detection threshold of 0.001. After age-class 2, reproductively effective reversion is the percentage of the population that reverts from triploidy to mosaicism (i.e. contains both triploid and diploid gamete cells) and therefore has the potential to reproduce. We conservatively assume that reverted triploids will have full reproductive capabilities, even though this has not yet been observed (Allen, unpubl. data). Default values for reproductively effective reversion were obtained from Calvo et al. (2001). The Mann and Evans (1998) fecundity model was modified to include reproductively effective reversion in the following way:

$$Frevert_{t,i} = 39.06 \times [0.000423 \times L_{t,i}^{1.17475}]^{2.36} \times N_{t,i} \times (R_{t,i} + T_{t,i}), \quad (2)$$

where $Frevert_{t,i}$ = total fecundity (number of eggs produced) for reverted triploid oysters at time *t* for age-class *i* greater than one;
$R_{t,i}$ = reproductively effective reversion rate at time *t* for age-class *i* greater than one; and
$T_{t,i}$ = diploid detection threshold at time *t* for age-class *i* greater than one.

The variable *Fs* is the effect of salinity on fecundity obtained by using the mean salinity value for the area in Chesapeake Bay where a particular population of Suminoe oysters is located. The value of *Fs* ranges from zero (meaning zero fecundity) to one (meaning no effect of salinity on fecundity). For the eastern oyster, when salinity is below 8.0 ppt, *Fs* is equal to zero, thereby making fecundity zero (Mann and Evans, 1998). When salinity is between 8.0 ppt and 13.5 ppt, there is a positive relationship between salinity and fecundity as described below:

$$Fs = \frac{(S-8)}{5.5}, \qquad (3)$$

where Fs = the effect of salinity on fecundity; and
S = salinity (ppt) between 8 ppt and 13.5 ppt.

When salinity is greater than 13.5 ppt, *Fs* is equal to one denoting no effect of salinity on fecundity. When salinity is greater than 35 ppt, *Fs* is equal to zero, making fecundity equal to zero (Mann and Evans, 1998). Low or no fertility at high salinity is apparently the case for *C. ariakensis* as well (Langdon and Robinson, 1996).

The variable for disease prevalence, F_d, has a value between 1.0 (no mortality from disease) and 0.0 (all oyster spat die from disease). Recent field studies have suggested that the Suminoe oyster is resistant to diseases on the east coast of North America (Calvo et al., 2001); therefore, the default value of *Fd* was set at one. Nevertheless, we retained this variable in the model to account for future data sets or other diseases so that the user can select F_d based on the prevalence of disease in the area to be modeled.

Oyster density is determined from the area over which the population occurs. Density affects gamete fertilization efficiency such that more dense oyster deployments (farms, reefs, etc.) exhibit an increased fertilization rate. Levitan (1991) reported the influence of body size and population density on fertilization success and reproductive output, and his equation was rewritten by Mann and Evans (1998) as

$$Ff_{t,i} = 0.0049 \times D_{t,i}^{0.72}, \qquad (4)$$

where $Ff_{t,i}$ = fertilization efficiency at time *t* for age-class *i* greater than one ranging from zero (meaning zero fertilization) to one (meaning all gametes become fertilized); and
$D_{t,i}$ = oyster density (number of oysters per square meter) at time *t* for age-class *i* greater than one.

For diploid oysters, oyster density is equal to the number of oysters in the population divided by the area (m^2). However, the density value for Equation 4 will differ with triploid populations because not all oysters may be able to reproduce; thus we modified the density equation to reflect the density of only undetected diploids and reverted triploids:

$$D_{t,i} = \frac{N_{t,i} \times (R_{t,i} + T_{t,i})}{A}, \qquad (5)$$

where A = area (square meters).

The variable for sex ratio, Fq_i, of females to males in the population per age class is a value from 0.0 to 1.0 (Mann and Evans, 1998). Fq_i modifies fecundity so that population size in Equations 1 and 2 comprised females only. The ratios of female-to-male Suminoe oysters at ages 1, 2, 3, and 4 are 0.28, 0.66, 1.00, and 1.00, respectively (Yingya et al., 1992).

Hence, total number of offspring produced per each age class at each time step modified with the previous variables (Mann and Evans, 1998) is as follows:

$$Ftotal_t = \sum (Frevert_{t,i} \times Fs \times Fq_i \times Fd \times Ff_{t,i}), \qquad (6)$$

where $Ftotal_t$ = modified total number of offspring produced at time *t* summed across all age classes.

The number of recruits obtained from the model that survive to the next time step, thereby becoming age class one, depends on the total number of offspring produced from reverted oysters in older age classes, daily mortality rate (ranging from 0.07 to 0.1) until settlement (21 days after fertilization) (Mann and Evans, 1998), the probability of successful completion of metamorphosis (0.25) (Mann and Evans, 1998), and total mortality for settled oysters less than one year old (Thorson, 1966). Hence, the number of individuals that will survive to enter age-class one at the next time step is given by the equation below:

$$N_{t,0} = K_t + \left(Ftotal_t \times \left(Pmet \times (1 - Lmort)^{21} \times (1 - M_0)\right)\right), \qquad (7)$$

where K_t = the number of oyster spat stocked at time *t*;
$Pmet$ = probability of successful completion of metamorphosis;
$Lmort$ = daily larval mortality rate until settlement at 21 days; and
M_0 = total mortality rate for settled oysters less then one year old.

The mortality variables in the model for adult oysters are natural mortality and harvest mortality. Natural mortality determines the proportion of oysters in each age class of the population from nonharvest causes each year. Default natural mortality rates were taken from Calvo et al. (2001) (Table 2). Stochastic values of these variables are chosen from a log normal distribution of the variance around the mean mortality rate for each age class of the population. Harvest-mediated mortality in the population was imposed by randomly selecting individuals for harvest in the age classes whose mean shell length is greater than the set minimum shell length-at-harvest. The harvest rate was a percentage of the population removed from the total population each year.

Certainty of harvest is defined as how certain we are that harvest occurs at a desired harvest rate. This variable in the model captures the effects of different harvest strategies. For example, if oysters are contained in wire cages, the certainty of obtaining a given harvest rate could be 100%. However, for oysters planted on the bottom, the certainty in obtaining a 100% harvest rate would be lower.

Population size for the current year is determined from the previous year's population size, harvest rate, natural mortality, and certainty in obtaining the desired harvest rate. Thus, the next year's starting population for the next age class greater than one is

$$N_{t+1,i+1} = N_{t,i} \times e^{-((H_i \times C)+M_{t,i})}, \qquad (8)$$

where H_i = harvest rate for age-class i greater than one;
C = certainty in obtaining the desired harvest rate; and
$M_{t,i}$ = natural mortality rate at time t for age-class i greater than one.

Total population size is determined by the summation of all individuals across all age classes:

$$Ntotal_t = \sum N_{t,i}, \qquad (9)$$

where $Ntotal_t$ = the total population size at time t for all age classes.

Model simulations and output

The model provides two options for output to the user. The first option shows results of one run of the simulation model. The output is a graph showing population size over time. The other output option shows the distribution of outcomes resulting from running the same scenario (i.e. the same input parameter and variable values) a set number of times. This output option shows the probability of the population becoming self-sustaining given the specified set of input conditions and yields probability profiles for risk assessment (Rosenburg and Restrepo, 1994). Probabilities range from 0%, meaning there is a zero probability of a population becoming self-sustaining given a set of input conditions, to 100%, meaning that this outcome will occur every time under the given set of input conditions.

Self-sustainability of a population is tested by running the simulation for a specified number of years with stocking, and then continuing to calculate population size for a specified number of additional years without stocking. Should the population size become zero, then the population is supported solely by stocking. However, should population size prove greater than the number stocked in earlier years, then the population is self-sustaining. The default setting for the simulation is to continue running the simulation twenty years without stocking, reflecting a maximum longevity of 20 years for Suminoe oyster (Cahn, 1950).

To understand the effects of changes in key variables on model predictions, we performed a sensitivity analysis.

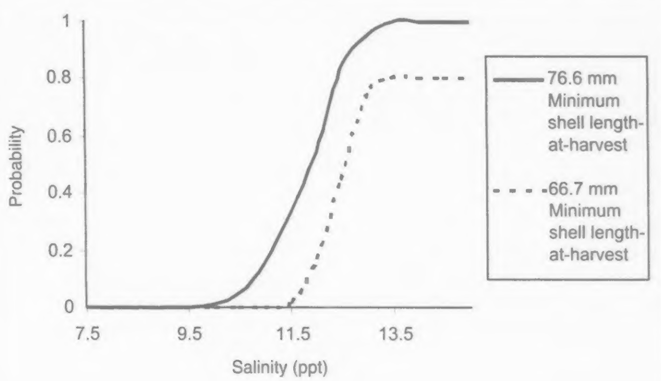


Figure 2

Relationship between salinity at deployment site and probability of a *C. ariakensis* population becoming self-sustaining, when area is set at 100 m^2, stocking size is set at 20,000 oyster, and when all other variables are set at default values. The solid line represents the relationship when the minimum shell length-at-harvest is 77.6 mm. The dashed line represents the relationship when the minimum shell length-at-harvest is 66.7 mm.

A first set of model runs changed the value of only one variable at a time, while keeping all other variables constant at default values. The variables that were changed were salinity, certainty of obtaining the desired harvest rate, minimum shell length-at-harvest, and stocking density. The second set of model runs was similar to the first, except changes were made to the values of two variables at a time while all other variables remained constant at default values. Salinity was varied from 8.5 ppt to 13.5 ppt. Certainty of obtaining the desired harvest rate was varied from 0.5 to 1.0. Minimum shell length-at-harvest was varied from 60 mm to 100 mm. To determine stocking density, the number of oysters stocked was varied from 100 to 1,000,000 oysters, but the area was set at 300 m^2.

All simulation results reported below were obtained by using default values for all variables (Table 2), unless noted otherwise.

Results

Effects of four major variables on the probability of a Suminoe oyster population becoming self-sustaining were examined by using the simulation model. These variables were salinity, certainty of obtaining the desired harvest rate, minimum shell length-at-harvest, and stocking density.

Salinity

Salinity between 8 ppt and 13.5 ppt affected the likelihood of developing self-sustaining populations because when salinity decreases, fecundity decreases (Fig. 2). However, this trend can be altered or masked by the effects of other variables on the probability of a population becoming self-sustaining. For example, when minimum shell length-at-harvest was lowered from 76.7 mm to 66.7 mm, oysters could be grown in higher salinity areas without increasing the probability of the population becoming self-sustaining (Fig. 2). By harvesting the oysters sooner, there is decreased likelihood of reproductively effective reversion.

Certainty in harvest rate

As the certainty of obtaining the desired harvest rate increased, the probability of the population becoming self-sustaining decreased (Fig. 3). For example, if oysters were grown on the bottom, inability to reliably recapture all individuals is such that the certainty of obtaining the desired harvest rate would be lower than if cages were used. Lower certainty of harvest would increase the likelihood of reproductively effective reversion among older individuals remaining on site, thereby increasing probabilities of both reproduction and the development of a self-sustaining population. In contrast, if oysters were kept in confinements such as wire cages, the certainty in obtaining the desired harvest rate would be high. As a result, this would decrease the likelihood of reproductively competent individuals remaining on site and giving rise to a self-sustaining population.

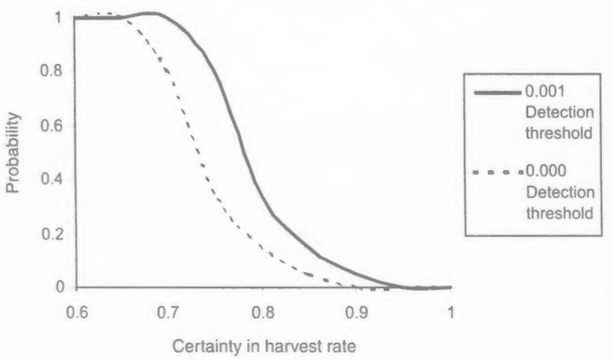


Figure 3

Relationship between the certainty in obtaining a specified harvest rate and probability of a *C. ariakensis* population becoming self-sustaining, when area is 300 m^2 and all other variables are set at default values. The solid line represents the relationship when the detection threshold for diploids is 0.001. The dashed line represents the relationship when the detection threshold for diploids is 0.000.

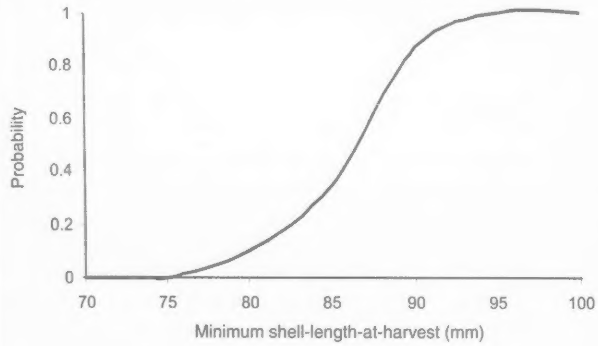


Figure 4

Relationship between minimum shell length-at-harvest (mm) and probability of a *C. ariakensis* population becoming self-sustaining, keeping all other variables at default values.

We also examined the relationship between certainty in obtaining the desired harvest rate and probability of a population becoming self-sustaining when the diploid detection threshold was zero, meaning 100% triploids were stocked (Fig. 3). This procedure distinguished between the effects of the technical problem of detection from the biological problem of reversion. It modeled the ideal scenario where flow cytometry detected any and all diploids in a batch but still allowed reproductively effective reversion to occur in age classes greater than two. When the detection threshold was set at zero and as certainty in obtaining the desired harvest rate increased, the probability of a population of triploid oysters becoming self-sustaining was decreased in relation to a detection threshold of 0.001. For example, when the detection threshold is 0.001 and certainty of harvest is set at 0.75, the probability of the population becoming self-sustaining is 0.082. In contrast, when the detection threshold is 0.000 and certainty of harvest is set at 0.75, the probability of the population becoming self-sustaining is 0.006.

Minimum shell-length-at-harvest

As the minimum shell-length-at-harvest was increased, the probability of the population becoming self-sustaining increased (Fig. 4). Increased probability of self-sustainability is due to the probability of reproductively effective reversion increasing the longer oysters are in the water, and the number of offspring produced by undetected diploids becoming higher.

Stocking density

With a 0.001 threshold for detecting diploids in triploid batches, and certainty of harvest of 0.9, the probability of the population becoming self-sustaining increased with increased stocking density (Fig. 5). Increased probability of self-sustainability is due to an increase in gamete fertilization efficiency as density increases (Mann and Evans, 1998). In contrast, when the diploid detection threshold was decreased to 0.000, meaning that all oyster spat stocked were indeed triploid, and certainty of harvest was maintained at the default value of 0.9, higher stocking densities barely increased the probability of the population becoming self-sustaining (Fig. 5). Absence of reproduction from undetected diploids, and the lack of reproduction in mosaic (reverted triploid) oysters until age 3, well past harvest size, were the principal determinants of lowered reproductive risk.

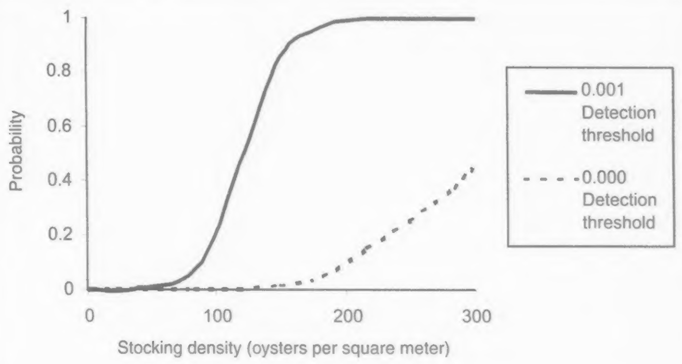


Figure 5

Relationship between stocking density and probability of a *C. ariakensis* population becoming self-sustaining, keeping all other variables at default values. The solid line represents the relationship when the detection threshold for diploids is 0.001. The dashed line represents the relationship when the detection threshold for diploids is 0.000.

Interaction of stocking density and salinity

At lower salinity sites, changes in stocking density had less of an effect on the likelihood of developing a self-sustaining population than at higher salinities when all other variables were set at default values (Fig. 6). Lower salinity decreases fecundity, which counteracts the increased fertilization efficiency at higher population densities.

Interaction of stocking density and certainty of harvest

Stocking density could be increased without increasing the risk of a self-sustaining population by increasing certainty of harvest, and keeping all other variables set at the default values (Fig. 7). Increased certainty of harvest decreased the number of oysters remaining in culture that were able to revert and reproduce, countering the increase in fertilization efficiency from increased density.

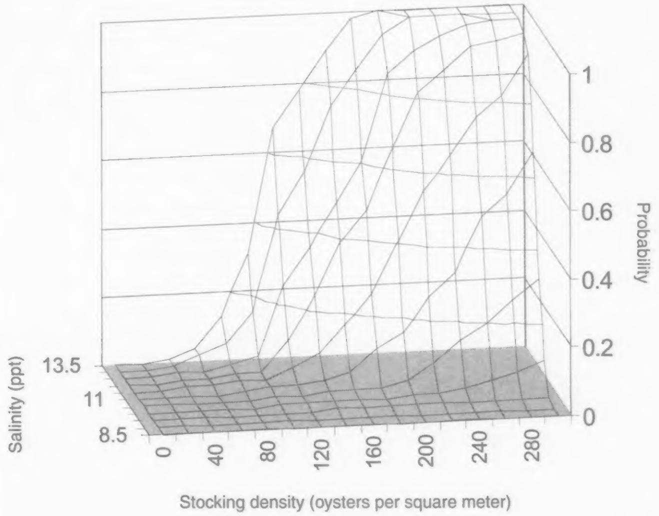


Figure 6

Relationship between stocking density, salinity at deployment site, and the probability of a *C. ariakensis* population becoming self-sustaining, keeping all other variables constant at default values and stocking area set at 300 square meters.

Discussion

The introduction of any non-native species into a new environment poses a number of potential ecological hazards. In general, these are related to two root causes: 1) the associated introduction of epibionts, pathogens, or other infectious agents, and 2) ecological disruption from the persistence of the introduced species (i.e. through reproduction, competition, etc.). To a large degree, associated introductions (with the possible exception of viruses) can be eliminated by adherence to codes of practice for proper quarantine and propagation, such as those set by the International Council for the Exploration of the Seas (ICES, 1994). The second risk of ecological disruption is caused by reproduction and subsequent naturalization. To address this hazard, sterile triploids have been proposed as a means to introduce the Suminoe oyster for commercial aquaculture. This model addresses those elements of risk associated with reproduction. Risk assessment modeling will enable managers to anticipate which management actions can have the greatest impact on decreasing the likelihood of a self-sustaining population. According to our results, risk reduction strategies include stocking Suminoe oysters in relatively low salinity, growing oysters in confinements (i.e. floating cages, lantern nets, bags, etc.) to maximize the certainty of achieving harvest, harvesting at the earliest possible (and presumably, economically feasible) opportunity, and maintaining a low population density of stocked oysters.

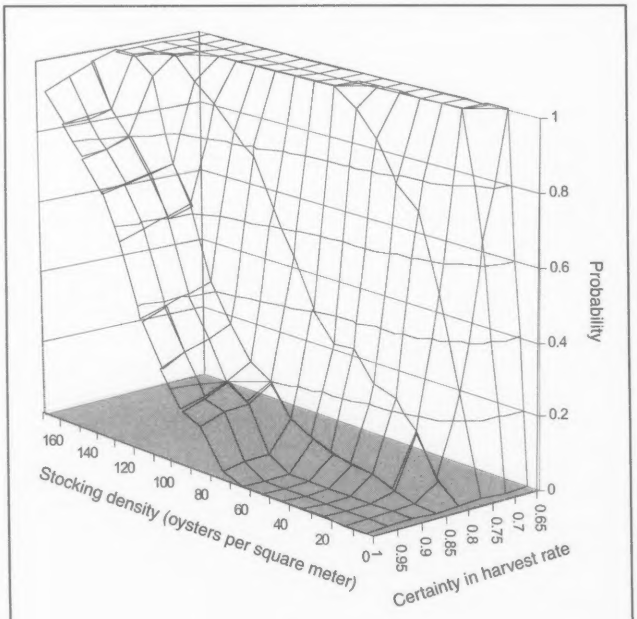


Figure 7

Relationship between stocking density, certainty in obtaining the desired harvest rate, and the probability of a *C. ariakensis* population becoming self-sustaining, keeping all other variables constant at default values and stocking area set at 300 square meters.

There are several key factors that affect the model's overall predictive value. First, few of the biological parameters that determine reproductive potential of Suminoe oyster are well known. All key parameters in the reproduction equations of the model were based on eastern oyster data (Mann and Evans, 1998) because of a lack of corresponding information about Suminoe oyster. For example, the parameter values in the equation for the relationship of oyster density and fertilization efficiency are not known for Suminoe oysters; therefore those for eastern oysters were used. It is important to determine these parameter values for Suminoe oyster so that the model may more accurately predict the relationship between density and the probability of the population becoming self-sustaining. Also, parameter values relating salinity and fecundity are unknown for Suminoe oyster; therefore eastern oyster parameter values were used. For most oyster species, gametogenesis is decreased or even nonexistent at lower salinities; however, the exact salinity value at which gametogenesis is decreased or absent may vary among species (Amemiya, 1929; Calabrese and Davis, 1970; Kennedy et al., 1996). The Suminoe oyster seems to thrive in estuarine conditions (Huang, 1962; Calvo et al., 2001) and there may be biological parallels to the eastern oyster. However, details about lower reproductive potential in lower salinity waters may be different for Suminoe oysters. In the wild, some populations of Suminoe oyster spawn in early spring in salinity as low as 10 ppt (Zhang and Lou 1956; Huang, 1962); therefore the model may slightly underestimate fecundity of Suminoe oyster in low salinity environments.

In this model, we also assumed that any oyster whose gamete cells reverted from triploid to "reproductive" mosaic or diploid recovered full fecundity. However, studies have yet to quantify recovered reproductive function in reverted triploids (Chandler et al., 1999). It is likely that reverted oysters would exhibit lower fecundity than diploid oysters because revertant oysters are mosaic; i.e. they comprise both triploid gamete-producing cells that are unable to produce viable gametes, and also diploid gamete-producing cells that are able to produce viable gametes. Continuing research with triploid Suminoe oyster should help us fill this gap in knowledge; however, until then, we decided that the model should err on the ecologically conservative side with the assumption that all reverted triploids exhibit full reproductive potential.

Despite its limitations, the model clearly points out key areas of concern, as well as highlights areas where more information or improvements in technology could prove critical. For example, advances in techniques for detecting very low proportions of diploids could reduce risk of a triploid Suminoe oyster population becoming self-sustaining under high stocking rates. Currently, flow cytometry is used for certifying triploid batches in subsampling larval populations (Allen and Bushek, 1992). Up to hundreds of thousands of larvae can be subsampled from a hatchery-scale larval culture. Cells disaggregated from triploid (and intermingled diploid) larvae then can be assayed. The difficulty lies in detecting extremely low levels of diploid cells within the mix. Improved detection by flow cytometry through repeated sampling techniques could help decrease the probability of stocking diploid individuals, thereby decreasing the subsequent chance for reproduction. Improved detection would also allow watermen to stock more Suminoe oysters in a smaller area.

We developed our own demographic model instead of using existing oyster models, such as the time-dependent, energy flow eastern oyster model (Hofmann et al., 1992; Dekshenieks et al., 1993; Hofmann et al., 1994; Powell et al., 1994; Powell et al., 1995; Dekshenieks et al., 1996; Powell et al., 1996; Ford et al., 1999) for various reasons. First, there were only two years of growth, mortality, and reversion rate data available for Suminoe oysters in the Chesapeake Bay (Calvo et al., 2001) and very little information in the literature about the Suminoe oyster in general. Hence, we decided that a demographics-based population dynamics model that tracked population size over time would be the most defensible method for achieving the objective of estimating the probability of a population becoming self-sustaining. Although the time-dependent energy flow model also tracks population size over time, all calculations are done in terms of energy, which then is converted into population size. Because the available Suminoe oyster data were demographic instead of bioenergetic, we felt a demographic population dynamics model made defensible use of available information. Additionally, the equations of the time-dependent energy flow model included parameters such as filtration rates, respiration, assimilation, and reproduction efficiency. These parameters have yet to be determined for the Suminoe oyster.

The scope of our model could be easily expanded to investigate more detailed scenarios and outputs. Economic aspects could be modeled to examine cost and profit and loss relationships for commercial production of triploid Suminoe oyster under various assumptions. In addition, the model could be adapted to a monthly instead of yearly time step, allowing managers to examine effects of stocking at different times of the year. The model could be made more spatially explicit, allowing managers to examine the probability of a population becoming self-sustaining over a larger area encompassing multiple deployment sites. Physical processes of water flow may have important effects on oyster recruitment. In our model, we assumed that water flow mimicked that in the James River, Virginia, as described in Mann and Evans (1998). Hence, our model is most appropriate for ecosystems where, as in the James River, larvae remain in the approximate area of their production. Clearly, this would not be the case at all sites. In sites where advection of larvae into or out of the area is at issue, one or more terms would have to be added to the model to account for such movement. Additional work is needed to assess a wider range of management options and potential risks.

Acknowledgments

This work was supported by the Virginia Sea Grant College Consortium. We are also grateful for support and help given by Gustavo Calvo, Patricia Flebbe, Rodger Mann, and Alison Williams.

Literature cited

Allen, S. K., Jr.
1983. Flow cytometry: assaying polyploidy fish and shellfish. Aquaculture 33:317–328.
Allen, S. K., Jr., and D. Bushek.
1992. Large scale production of triploid oysters *Crassostrea virginica* using "stripped" gametes. Aquaculture 103:241–251.
Amemiya, I.
1929. Notes on experiments on the early development stages of the Portuguese, American, and English native oysters, with special reference to the effect of varying salinity. J. Mar. Biol. Assoc. U.K. 14:161–175.
Cahn, A. R.
1950. Oyster culture in Japan, 80 p. Office of Technical Services report 134. U.S. Dep. Commer., Washington, DC.
Calabrese, A., and H. C. Davis.
1970. Tolerances and requirements of embryos and larvae of bivalve mollusks. Helgol. Wiss. Meeresunters. 20: 553–564.
Calvo, G. W., M. W. Luckenbach, S. K. Allen, and E. M. Burreson.
1999. Comparative field study of *Crassostrea gigas* and *Crassostrea virginica* in relation to salinity in Virginia. J. Shellfish Res. 18:465–473.
2001. Comparative field study of *Crassostrea ariakensis* and *Crassostrea virginica* in relation to salinity in Virginia. J. Shellfish Res. 20:221–229.
Chandler, W., A. Howe, and S. K. Allen Jr.
1999. Mosaicism of somatic and gametic tissues in *Crassostrea gigas* and *Crassostrea ariakensis*. J. Shellfish Res. 18:293.
Dekshenieks, M. M., E. E. Hofmann, and E. N. Powell.
1993. Environmental effects on the growth and development of eastern oyster, *Crassostrea virginica*, larvae: a modeling study. J. Shellfish Res. 12:241–254.
Dekshenieks, M. M., E. E. Hofmann, J. M. Klinck, and E. N. Powell.
1996. Modeling the vertical distribution of oyster larvae in response to environmental conditions Mar. Ecol. Prog. Ser. 137:97–110.
Ford, S. E., E. Powell, J. Klinck, and E. Hofmann.
1999. Modeling the MSX parasite in eastern oysters (*Crassostrea virginica*) populations. I. Model development, implementation, and verification. J. Shellfish Res. 18: 475–500.
Gottlieb, S. J., and M. E. Schweighofer.
1996. Oysters and the Chesapeake Bay ecosystems: a case for exotic species introduction to improve environmental quality? Estuaries 19:639–650.
Guo, X., and S. K. Allen Jr.
1994a. Reproductive potential and genetics of triploid *Crassostrea gigas*. Biol. Bull. 187:309–318.
1994b. Viable tetraploids in the Pacific oyster (*Crassostrea gigas* Thunberg) produced by inhibiting polar body I in eggs from triploids. Mol. Mar. Biol. Biotechnol. 3:42–50.
1997. Sex and meiosis in autotetraploid Pacific oyster, *Crassostrea gigas* (Thunberg). Genome 40:397–405.
Guo, X., G. A. DeBrosse, and S. K. Allen Jr.
1996. All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. Aquaculture 142:149–161.
Hallerman, E. M., and A. R. Kapuscinski.
1995. Incorporating risk assessment and risk management into public policies on genetically modified finfish and shellfish. Aquaculture 137:9–17.
Hallerman, E. M., M. Leffler, S. Mills, and S. K. Allen Jr.
2002. Aquaculture of triploid *Crassostrea ariakensis* in Chesapeake Bay: a symposium report. Maryland Sea Grant, Univ. Maryland, College Park, MD. http://www.mdsg.umd.edu/oysters/exotic/workshops.html.
Hofmann, E. E., E. N. Powell, J. M. Klinck, and E. A. Wilson.
1992. Modeling oyster populations III. Critical feeding periods, growth, and reproduction. J Shellfish Res. 11: 399–416.
Hofmann, E. E., J. M. Klinck, E. N. Powell, S. Boyles, and M. Ellis.
1994. Modeling oyster populations II. Adult size and reproductive effort. J. Shellfish Res. 13:165–182.
Huang, J.
1962. Study on the major cultivated species of oyster in Guangdong province, p. 41–52. *In* Transactions of the annual meeting of Oceanology and Limnology Society of Guangdong, Guangdong, China. ICES (International Council for the Exploration of the Seas), Palgade 2-4. DK-1261, Copemhagen K. Denmark.

1994. Codes of practice on the introduction and transfer of marine organisms, 17 p. Cooperative Research Report 204. ICES, Palgade 2-4. DK-1261, Copemhagen K. Denmark.

Kennedy, V. S., R. I. Newell, and A. F. Eble.
1996. The eastern oyster: *Crassostrea virginica*, 734 p. Maryland Sea Grant, Univ. Maryland, College Park, MD.

Lackey, R. T.
1994. Ecological risk assessment. Fisheries 19:14–18.

Langdon, C. J., and A. M. Robinson.
1996. Aquaculture potential of the Suminoe oyster (*Crassostrea ariakensis*). Aquaculture 144:321–338.

Levitan, D. R.
1991. Influences of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. Biol. Bull. 181:261–268.

Lipton, D. W., E. F. Lavan, and I. E. Strand.
1992. Economics of molluscan introductions and transfers: the Chesapeake Bay dilemma. J. Shellfish Res. 11:511–519.

Mann, R., E. M. Burreson, and P. K. Baker.
1991. The decline of the Virginia oyster fishery in Chesapeake Bay: considerations for the introduction of a nonendemic species, *Crassostrea gigas*. J. Shellfish Res. 10: 379–388.

Mann, R., and D. A. Evans.
1998. Estimation of oyster, *Crassostrea virginica*, standing stock, larval production, and advective loss in relation to observed recruitment in the James River, Virginia. J. Shellfish Res. 17:239–253.

Powell, E. N., J. M. Klinck, and E. E. Hofmann.
1996. Modeling diseased oyster populations. II. Triggering mechanisms for *Perkinsus marinus* epizootics. J. Shellfish Res. 15:141–165.

Powell, E. N., J. M. Klinck, E. E. Hofmann, and S. M. Ray.
1994. Modeling oyster populations. IV: Rates of mortality, population crashes, and management. Fish. Bull. 92: 347–373.

Powell, E. N., J. M. Klinck, E. E. Hofmann, E. A. Wilson-Ormond, and M. S. Ellis.
1995. Modeling oyster populations. V. Declining phytoplankton stocks and the population dynamics of American oyster (*Crassostrea virginica*) populations. Fish. Res. 24: 199–222.

Rosenberg, A. A., and V. R. Restrepo.
1994. Uncertainty and risk evaluation in stock assessment advice for U.S. marine fisheries. Can. J. Fish. Aquat. Sci. 51:2715–2720.

Thorson, G.
1966. Some factors influencing the recruitment and establishment of marine benthic communities. Neth. J. Sea Res. 3:267–293.

Yingya, C., D. Chenmao, and L. Zhigang.
1992. Studies on the ecology of *Crassostrea rivularis* in Zhanjiang Bay. Tropic. Oceanogr. 11:38–44.

Zhang, X., and Z. Lou.
1956. The oyster. Bull. Biol. 2:27–32. [In Chinese.]

Zhou, M.-F.
2002. Chromosome set instability in 1–2 year old triploid *Crassostrea ariakensis* in multiple environments. M.S. thesis, 72 p. Virginia Institute of Marine Science, Gloucester Point, VA.

Allozyme and RAPD variation in the eastern Pacific yellowfin tuna (*Thunnus albacares*)

Pindaro Díaz-Jaimes
Instituto de Ciencias del Mar y Limnología
Universidad Nacional Autónoma de México
Circuito exterior de Ciudad Universitaria
Apdo. Postal 70-305
México, D.F. 04510
E-mail address: pindaro@mar.icmyl.unam.mx

Manuel Uribe-Alcocer
Instituto de Ciencias del Mar y Limnología
Universidad Nacional Autónoma de México
Circuito exterior de Ciudad Universitaria
Apdo. Postal 70-305
México, D.F. 04510

Abstract—Stock structure of eastern Pacific yellowfin tuna was investigated by analyzing allozymes and random amplified polymorphic DNAs (RAPDs) from 10 samples of 20–30 individuals each, collected between 1994 and 1996 from fishing vessels operating in the Inter-American Tropical Tuna Commission (IATTC) yellowfin regulatory area (CYRA). Allozyme analysis resolved 28 loci, eight of which were polymorphic under the 0.95 criterion: *Aat-S**, *Glud*, *Gpi-F**, *Gpi-S**, *La*, *Lgg*, *Pap-F**, and *6-Pgd*, resulting in a mean heterozygosity over all allozyme loci of $H = 0.052$. Four polymorphic RAPD loci were selected for analysis, resulting in a mean heterozygosity of $H = 0.43$. Eight of 45 pairwise comparisons of allozyme allele frequencies among the ten samples showed significant differences after correction for multiple testing ($P<0.0001$), all of which involved comparisons with the Gulf of California sample. Confirmation of this signal of population structure would have management implications. No significant divergence in RAPD allele frequencies was observed among samples. Weir and Cockerham θ estimated for allozyme loci ($\theta=0.048$; $P<0.05$) and RAPD loci ($\theta=0.030$; $P>0.05$) revealed little population structure among samples. Mantel tests demonstrated that the genetic relationships among samples did not correspond to an isolation-by-distance model for either class of marker. Four of eight comparisons of coastal and offshore samples revealed differences of allele frequencies at the *Gpi-F** locus ($P<0.05$), although none of these differences was significant after correction for multiple testing ($P>0.001$). Results are consistent with the hypothesis that the CYRA yellowfin tuna samples comprise a single genetic stock, although gene flow appears to be greater among coastal samples than between coastal and offshore samples.

Manuscript approved for publication 19 June 2003 by Scientific Editor.
Manuscript received 26 June 2003 at NMFS Scientific Publications Office.
Fish. Bull. 101:769–777.

Yellowfin tuna (*Thunnus albacares*) is a cosmopolitan species inhabiting tropical and subtropical waters in the Atlantic, Pacific, and Indian oceans. This species has accounted for more than a third of the world's tuna production since 1970. The eastern Pacific has contributed from 21% to 26% of the global catch from 1993 through 1997, representing 273,329 metric tons (t) in 1990 to 264,426 t in 1998 (IATTC, 1999).

Yellowfin tuna is a large pelagic fish with a common size of 150 cm (Collette and Nauen, 1983). Spawning occurs throughout the year in the tropical oceans, preferably near islands and coasts (Leis et al., 1991). Growth is rapid and individuals reach maturity by the end of the second year (Suzuki et al., 1978). Schooling of individuals of similar size is observed near surface waters and is often associated with floating objects (Wild, 1994).

Yellowfin tuna is currently considered to comprise a single species (Gibbs and Collete, 1967), although significant morphometric and meristic differences, limited fish movements, and differences among catch data, have been reported for the different regions of the Pacific Ocean (Godsil and Greenhood, 1951; Schaefer, 1955; Joseph et al., 1964; Suzuki et al., 1978; Schaefer, 1991). Population structure in yellowfin tuna has been addressed in the Pacific Ocean by using several independent methods. Morphometric and meristic based studies have shown significant differences (Godsil and Greenhood, 1951; Schaefer, 1955; Kurogane and Hiyama, 1957), and at least three stocks or discrete units (western, central, and eastern Pacific) have been proposed. More recent studies using morphometric multivariate analysis suggest the presence of different stocks between north and south regions in the eastern Pacific (Schaefer, 1991), as well as across the Pacific Ocean (Schaefer, 1991). Additionally, differences in larval distribution, catch rates, and size composition data of yellowfin tuna caught along the equatorial Pacific by longline and purse-seine have been used by Suzuki et al. (1978) to distinguish between western, central, and eastern Pacific groups.

Tagging experiments have shown limited movement of yellowfin tuna between western and eastern Pacific waters (Joseph et al., 1964; Fink and Bayliff, 1970). In the eastern Pacific, the presence of two groups has been suggested: a northern group off Baja California coast and the Revillagigedo Islands and a southern group from the Maria Islands through Chile. Some mixing occurs between them (Fink and Bayliff, 1970). There seem to be marked movements between north and south groups along the coast with limited westward movements (Joseph et al., 1964).

Some studies of population structure using genetic analyses have not revealed the presence of discrete stocks along the Pacific Ocean. Barret and Tsuyuki (1967) used transferrin analysis and did not find differences in allele frequencies between samples from Hawaii and eastern Pacific samples, although heterogeneity was detected within the eastern Pacific samples (IATTC, 1975). Allozyme variation studies in the esterase locus (Fujino, 1970) did not show enough evidence of genetic differentiation between eastern Pacific and Hawaii samples. Furthermore, Scoles and Graves (1993) used restriction fragments length polymorphisms (RFLP) and analysis of mitochondrial (mt) DNA to examine five samples collected across the Pacific Ocean and one from the Atlantic Ocean. Although they detected 34 haplotypes and considerable genetic variation, no evidence of genetic differentiation among samples was found.

However, more recent genetic studies have provided limited evidence of genetic heterogeneity. Ward et al. (1994) analyzed four polymorphic allozyme loci and 18 mtDNA haplotypes in yellowfin tuna from the Pacific Ocean. Although no unique haplotypes were found in the analyzed populations through RFLP analysis, the eastern Pacific samples were found to be different from the central and western Pacific samples in frequency differences at a single locus *GPI-F**, suggesting that the signal of population structure exhibited is due to selective factors contributing to the divergence. Eastern Pacific samples (n=41) were collected in the northeast Pacific off California and at an unspecified site off Mexico (n=40). Comparisons of *GPI-F** allele frequencies from eastern Pacific also included two samples previously analyzed by Sharp (1978) from Roca Partida (Central America) and Ecuador. Their results showed population homogeneity at the *GPI-F** locus for this region.

To date, the methods and logistics used to study divergence in the Pacific yellowfin tuna have been focused on a global rather than a local scale, and sampling has been focused on the wide areas of the west and central Pacific. Local structure in the eastern Pacific yellowfin tuna has not been addressed through a more intense sampling strategy to examine genetic homogeneity in this region. Because tagging studies have shown restricted longitudinal movements by yellowfin tuna, population structure and isolation by distance hypotheses can be tested. To evaluate the stock structure of yellowfin tuna in eastern Pacific, we employed analyses of allozymes and of randomly amplified polymorphic DNA (RAPDs).

RAPDs have proven to be useful genetic markers because of their high levels of polymorphisms (Williams et al., 1990; Welsh et. al., 1991). They have been used to estimate population structure in fishes, including the cod (Kenji, 1998), red mullet (Mamuris et al., 1998), and striped bass (Bielawski and Pumo, 1997). The use of RAPDs, considered as neutral markers, and the simultaneous use of allozyme analyses with intense sampling in a more local area, might provide evidence about the relationship between gene flow and spatial distribution of the eastern Pacific yellowfin tuna, as well as evidence of the presence of local selective factors responsible for the divergence suggested by Ward et al. (1994).

Materials and methods

Sampling

A total of 327 tissue samples from specimens of ten locations were obtained from commercial tuna boats fishing in the tropical eastern Pacific from 1994 to 1996 (Fig. 1). Muscle tissue samples were dissected from specimens at the time of landing and were transported in liquid nitrogen or on dry ice to the Laboratorio de Genética de Organismos Acuáticos of the Instituto de Ciencias del Mar y Limnología in Mexico City. Samples were maintained at –70°C until processing.

Allozyme analysis

For allozyme analysis, 1 cm^3 (about one gram) of tissue sample was ground with a manual homogenizer in 1.5 mL of extraction buffer (0.01M Tris-0.001M EDTA, pH 6.8, and 1% NADP) and centrifuged at 2500 g at 4°C. Electrophoretic runs were performed in 12% (w/v) starch gels (Sigma Chemicals, St. Louis, MO). Four buffer systems were used to analyze nineteen enzymes that resolved 28 loci, eight of which showed polymorphism: *Aat-S** (aspartate aminotransferase), *Glud* (glutamate dehydrogenase), *Gpi-F** and *Gpi-S** (glucose phosphate isomerase), *La* (leucil-L-alanine), *Lgg* (L-leucil-glycil-glycine), *Pap-F** (L-leucil-L-proline) and *6-Pgd* (phosphogluconate dehydrogenase). Enzymes AK (adenilate kinase), CK (creatinine kinase), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), LDH (lactate dehydrogenase), MDH (mMalate dehydrogenase), ME (malic enzyme) and SOD (superoxide dismutase), displayed twenty more loci that were presumably monomorphic. Buffer systems for enzyme analysis were 1) amino-citrate: 0.04M citric acid, 15mL/L of N-3-aminopropyl-morpholine, pH 6.5 (AAT, GPI, and LA); 2) 0.008M Tris, 0.003 M citric acid, pH 6.7 (GLUD and 6-PGD); 3) 0.025 M Tris, 0.192 glycine, pH 8.5 (GPI and LGG); 4) 0.0.076 M Tris, 0.005 M citric acid, pH 8.7 (PAP). Enzyme assays were performed following Harris and Hopkinson (1976). Enzymes showing polymorphism were analyzed for all samples and subjected to population genetic analysis.

RAPD analysis

For RAPD analyses, genomic DNA was extracted from muscle tissue by using standard phenol-chloroform protocols (Sambrook et al., 1989), resuspended in TE buffer (10mM Tris-0.1mM EDTA pH 8.0), and quantified with a Hoefer DyNA quant 200 fluorometer. DNA was amplified with primer F-10 (Operon® Alameda, CA; 5′-GGAAGCTTGG-3′). Amplifying reactions were performed in a final volume of 22 μL consisting of 0.7 to 1 ng/μL of DNA in amplification buffer, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 33 ng of primer, 10 mM dNTPs, and 1 U of Taq polymerase. Amplification of genomic DNA was performed in a Perkin Elmer®, Foster City, CA (mod. 480), thermal cycler. The program was set for 1 cycle of 1 min. at 36°C, followed by 44 cycles of 1 min. at 36°C; 1 min. at 94°C; 2 min. at 72°C, and

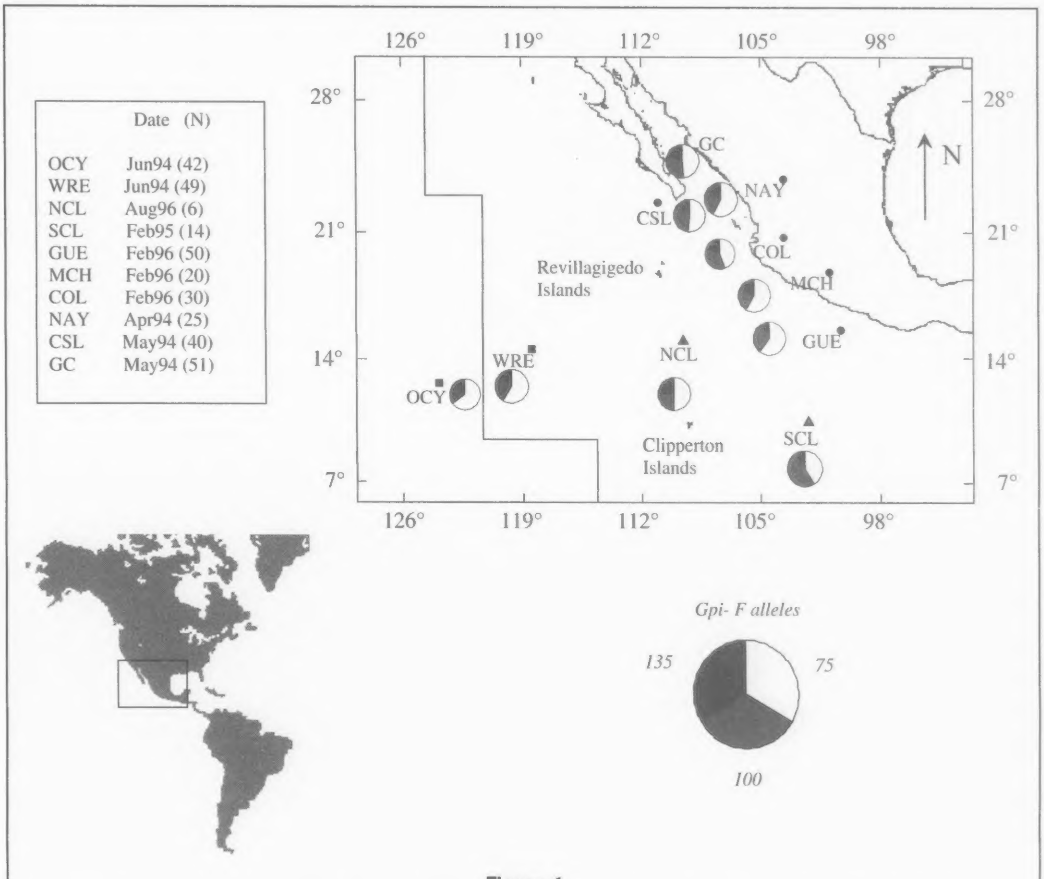

Date (N)
OCY Jun94 (42)
WRE Jun94 (49)
NCL Aug96 (6)
SCL Feb95 (14)
GUE Feb96 (50)
MCH Feb96 (20)
COL Feb96 (30)
NAY Apr94 (25)
CSL May94 (40)
GC May94 (51)
126°
119°
112°
105°
98°
28°
21°
14°
7°
N
GC
NAY
CSL
COL
MCH
GUE
Revillagigedo Islands
NCL
WRE
OCY
Clipperton Islands
SCL
Gpi- F alleles
135
75
100

Figure 1

Thunnus albacares. Location of sampling sites, dates, and sample sizes, and *Gpi-F** gene frequencies of eastern Pacific yellowfin tuna. The sites are as follows: OCY = out of CYRA area; WRE = west of the Revillagigedo Islands; NCL = North Clipperton islands; SCL = South Clipperton Islands; MCH = Michoacan; GUE = Guerrero; COL = Colima; CSL = Cape San Lucas; NAY = Nayarit; GC = Gulf of California. Symbols are ● coastal, ▲ intermediate, ■ offshore.

a final cycle of 15 min. at 72°C. Optimal DNA concentrations for amplification were determined by testing several dilutions, one of which was taken as the standard for every subsequent amplification.

Amplified fragments were resolved by electrophoresis in 1.5% agarose gels (Sigma Chemicals) for 3 to 4 h. at 90 mA (100 V). A 100bp DNA Ladder (GibcoBRL, Gaithersburg, MD, 15628-019) was used as size standard. After electrophoresis, gels were stained with ethidium bromide and photographed in a UV light transilluminator.

Data analysis

Allelic frequencies, test of conformity of genotype distributions with Hardy-Weinberg, and heterozygous deficit were determined by using Genepop version 3.3 (Raymond and Rousset[1]). Homogeneity of allozyme and RAPD allele frequencies was evaluated by using the exact probability test (Raymond and Rousset, 1995) consisting of a contingency analysis for every polymorphic locus and an estimation of their probability values by the combined probability of Fisher (Sokal and Rohlf, 1995) as implemented in the TFPGA program (Miller[2]). Pairwise comparisons were conducted to determine allele frequency differences among samples in order to define sources of variation. Based on the longitudinal differentiation pattern observed by Ward et al. (1994) and the morphological latitudinal differences within eastern Pacific samples reported by Schaefer (1991) at north-south of the 15–20°N range, spatial homogeneity was tested at the following levels: overall samples (O), among longitudinal regions (L; coastal, intermediate, and offshore localities), and latitudinal regions (N; north-south of the 15–20° range). The Gulf of California sample was excluded from this analysis because the large variation found in this sample (reflected in the significant differences shown in allele frequency homogeneity from pairwise comparisons) would not allow an accurate assessment of whether longitudinal or latitudinal differentiation exists or not. Significance levels were adjusted for multiple testing through the Bonferroni sequential method (Rice, 1989).

---

[1] Raymond, M. L., and F. Rousset. 1995a. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicist. J. Heredity 86:248–249.

[2] Miller, M. P. 1997. Tools for genetic populations analyses (TFPGA) 1.3: a windows program for the analysis of allozyme and molecular population genetic data, 29 p. Computer software distributed by the author at http://bioweb.usu.edu/mpmbio.

Population subdivision was estimated by using the Weir and Cockerham (1984) method through the TFPGA program. Standard error and confidence intervals were obtained through jackknife and bootstrapping procedures, respectively, with F_{ST} Pro 1.0 (Weir, 1990). Estimates of population subdivision were partitioned into the following levels: O, over all the samples; L, longitudinal regions (coastal, intermediate and offshore); and N, latitudinal regions.

We used the θ statistic to estimate gene values between sample pairs (Slatkin, 1993) that are defined as M_θ. An "isolation by distance" model was evaluated from the correlation between the distance between localities measured as geographic separation in nautical miles (nmi), and the M_θ values by means of the Mantel test (Hellberg, 1994) in both allozymes and RAPDs.

The patterns of the amplification products resulting from the RAPD analysis were subjected to the same analyses as allozymes with the procedures described in Lynch and Milligan (1994). RAPD fragments were interpreted under the following assumptions: 1) fragments were considered to behave as dominant genes (Williams et al., 1990); 2) every polymorphic fragment was considered derived from a two-allele locus; 3) the equilibrium of Hardy-Weinberg was assumed for all genotypes, and 4) each fragment was considered to be an independent locus.

Only those fragments clearly defined and having consistent intensity were recorded. Because of this, the Michoacan sample, with poor consistency in the banding patterns, was excluded from the RAPD analysis. The allele frequency of every fragment was calculated on the basis of the inferred homozygous recessive genotypes. Because of the dominant nature of the alleles, and in order to correct the bias originated by calculating the recessive allelic frequencies, we chose the estimation based on the Taylor expansion (Kendall and Stuart, 1977, cited in Le Corre et al., 1997) as implemented in TFPGA program. This reduction on the bias is based on the equation resulting from the second order expansion of Taylor (see details in Lynch and Milligan, 1994).

Results

Allozymes

Of the 28 analyzed allozyme loci, eight (28.5%) showed polymorphism under the 0.95 criterion. The observed heterozygosities per sample over all allozyme loci ranged from 0.027 to 0.083 (mean 0.052). Allozyme frequencies for the eight polymorphic loci detected are shown in Table 1. After adjusting levels of significance by the Bonferroni procedure, significant deviation of genotypic frequencies from those expected under Hardy-Weinberg was found in the loci *Lgg* and *Pap-F** for the Gulf of California sample and in the *Aat-S** locus for two localities—west of Revillagigedo Islands, and the Gulf of California ($P<0.0006$, Table 1). Deviations displayed for both locations corresponded to a heterozygous deficit ($P<0.0006$, after Bonferroni correction).

Comparison of allozyme allele frequencies among all collections (overall) by the exact probability test revealed significant heterogeneity at loci *Glud*, *La*, and *Lgg*, after Bonferroni adjustment ($P<0.006$). Pairwise comparisons among samples to test allele homogeneity gave significant differences for nine of 45 comparisons after correction for multiple tests ($P<0.001$), seven of which involved comparisons with the Gulf of California (GC) sample. The remaining significant differences were between Guerrero-Nayarit, and Cape San Lucas-Nayarit comparisons (Table 2), resulting from significant heterogeneity at *Glud*, *La*, and *Lgg* loci.

In general, allozyme analysis displayed low levels of differentiation. The θ value over all loci was different from zero ($P<0.05$) and showed that 4.8% of the variance was attributable to differences among samples (Table 3).

Individual loci showed θ values ranging between 0.0037 and 0.27. Highly significant values at loci *La* (θ=0.13 ±0.089) and *Lgg* (θ=0.27 ±0.253) evidently resulted from their weak polymorphism in some samples (Table 3).

Allele frequency homogeneity was tested among coastal, intermediate, and offshore regions (Table 4). Significant heterogeneity was found by exact test between coastal and offshore comparisons ($P=0.0043$) but was found to be not significant between coastal and intermediate regions ($P=0.0632$). Subdivision as measured by θ among coastal, intermediate, and offshore localities was not different from zero. However, for the *Gpi-F** locus the population subdivision among regions (0.0058) was twice as large as that noted among samples (θ=0.003), but neither value was significant (Table 3). No latitudinal differentiation by the exact test or population subdivision estimated by the θ index was found between north and south regions (data not shown).

The gene flow values (M_θ) were high (mean 24.8 migrants per generation). A lack of correlation between gene flow estimations and geographic distance by means of the Mantel test was observed (r^2=–0.144; P=0.22), resulting in a rejection of the isolation by distance model.

For the *Gpi-F** locus, paired tests of significance (data not shown) showed discrepancies in the *Gpi-F*/75* allele frequencies among localities from the coast with those located at the CYRA limits (Fig. 1, Table 5). Four of eight comparisons of coastal and offshore samples revealed differences of allele frequencies at this locus ($P<0.05$), although none of these differences was significant after correction for multiple testing ($P>0.001$).

RAPDs

The primer OPF-10 produced 11 amplified fragments, with sizes from 200 to 600 bp (base pairs). Four of the fragments

Table 1

Allele frequencies for allozymes and RAPDs, samples size (n) and agreement to the Hardy-Weinberg equilibrium (HW) for every loci and sample of *Thunnus albacares*. Significance values for HW tests were adjusted for multiple comparisons with an initial α level of 0.0006 [(0.05/(8 loci × 10 samples)]. P = probability of significance for allele frequency heterogeneity per locus, * = significant at α = 0.006. — = no data. OCY = out of CYRA area; WRE = west of the Revillagigedo Islands; NCL = North Clipperton islands; SCL = South Clipperton Islands; MCH = Michoacan; GUE = Guerrero; COL = Colima; CSL = Cape San Lucas; NAY = Nayarit; GC = Gulf of California.

		Collection										
Locus	Allele	OCY	WRE	NCL	SCL	GUE	MCH	COL	CSL	NAY	GC	*P*
*Aat-S**	–90	0.171	0.266	0.250	0.231	0.154	0.275	0.077	0.092	0.0	0.234	
	–100	0.829	0.734	0.750	0.769	0.846	0.725	0.923	0.908	1.0	0.766	
	n	41	32	6	13	39	20	26	38	25	47	0.011
	HW	yes	no	yes	yes	yes	yes	yes	yes	—	no	
Glud	100	0.700	0.634	0.667	0.857	0.663	0.775	0.914	0.788	0.680	0.395	
	85	0.300	0.366	0.333	0.143	0.337	0.225	0.086	0.212	0.320	0.605	*
	n	40	41	6	14	49	20	29	40	25	43	
	HW	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	
*Gpi-F**	135	0.071	0.134	0.250	0.091	0.190	0.079	0.185	0.125	0.160	0.200	
	100	0.262	0.280	0.250	0.500	0.230	0.316	0.370	0.363	0.260	0.314	
	75	0.667	0.586	0.500	0.409	0.580	0.605	0.444	0.512	0.580	0.486	0.526
	n	42	41	6	11	50	19	27	40	25	35	
	HW	yes	yes	yes	yes	yes	no	yes	yes	yes	yes	
*Gpi-S**	–60	0.402	0.536	0.417	0.571	0.360	0.425	0.414	0.449	0.479	0.338	
	–100	0.598	0.464	0.583	0.429	0.640	0.575	0.586	0.551	0.521	0.663	0.015
	n	41	42	6	14	50	20	29	39	24	40	
	HW	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	
La	120	0.0	0.024	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	
	100	1.0	0.976	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.8	*
	n	42	42	6	14	50	20	24	40	20	50	
	HW	yes	—	—	—	—	—	—	—	yes	yes	
Lgg	115	0.0	0.024	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.349	
	100	1.0	0.976	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.651	*
	n	42	42	6	14	50	20	24	24	24	43	
	HW	yes	—	—	—	—	—	—	—	—	no	
*Pap-F**	110	0.0	0.0	0.0	0.0	0.02	0.05	0.0	0.0	0.0	0.083	
	100	1.0	1.0	1.0	1.0	0.98	0.95	1.0	1.0	1.0	0.917	0.055
	n	42	42	6	14	50	20	29	40	25	48	
	HW	—	—	—	—	yes	yes	—	—	—	no	
6-Pgd	100	0.902	0.903	0.667	0.821	0.939	1.0	0.966	1.0	0.840	0.938	
	90	0.098	0.097	0.333	0.179	0.061	0.0	0.034	0.0	0.160	0.062	0.007
	n	41	31	6	14	49	20	29	40	25	48	
	HW	yes	yes	yes	yes	yes	—	yes	—	yes	yes	
F10-1	*a*	0.406	0.282	0.234	0.322	—	0.083	0.505	0.355	0.282	0.246	
	b	0.594	0.718	0.766	0.678	—	0.917	0.495	0.645	0.718	0.754	0.572
	n	9	8	7	9	—	6	9	10	8	9	
	HW	—	—	—	—	—	—	—	—	—	—	
F10-2	*a*	0.406	0.603	0.441	0.322	—	0.278	0.322	0.355	1.0	0.322	
	b	0.594	0.397	0.559	0.678	—	0.722	0.678	0.645	0.0	0.678	0.369
	n	9	8	7	9	—	6	9	10	8	9	
	HW	—	—	—	—	—	—	—	—	—	—	
F10-3	*a*	0.406	0.475	0.329	0.625	—	0.397	0.406	0.529	0.371	0.406	
	b	0.594	0.525	0.671	0.375	—	0.603	0.594	0.471	0.629	0.594	0.912
	n	9	8	7	9	—	6	9	10	8	9	
	HW	—	—	—	—	—	—	—	—	—	—	
F10-4	*a*	0.115	0.282	0.234	0.178	—	0.083	0.246	0.219	0.138	0.246	
	b	0.885	0.718	0.766	0.822	—	0.917	0.754	0.781	0.862	0.754	0.929
	n	9	8	7	9	—	6	9	10	8	9	
	HW	—	—	—	—	—	—	—	—	—	—	

Table 2

Pairwise-sample comparisons of allele frequency homogeneity for *Thunnus albacares*. Probability values in allozymes are above the diagonal (—) and RAPDs are below the diagonal (—). * = significant values after Bonferroni correction for multiple tests (initial α was 0.05). na = data not available. OCY = out of CYRA area; WRE = west of the Revillagigedo Islands; NCL = North Clipperton islands; SCL = South Clipperton Islands; MCH = Michoacan; GUE = Guerrero; COL = Colima; CSL = Cape San Lucas; NAY = Nayarit; GC = Gulf of California.

Sample	OCY	WRE	NCL	SCL	GUE	MCH	COL	CSL	NAY	GC
OCY	—	0.5807	0.7391	0.3414	0.4386	0.8254	0.0295	0.1525	0.0194	*<0.001
WRE	0.9316	—	0.9482	0.6047	0.4330	0.1180	0.0012	0.0084	0.0192	*<0.001
NCL	0.9797	0.9770	—	0.9232	0.3494	0.7307	0.1567	0.0859	0.4779	0.0174
SCL	0.9798	0.8088	0.8915	—	0.3989	0.0717	0.3414	0.1093	0.0287	*<0.001
GUE	0.5935	0.4732	0.9095	0.7757	—	0.4137	0.1723	0.5932	*<0.001	*<0.001
MCH	na	na	na	na	na	—	0.0500	0.1532	0.0096	*<0.001
COL	0.9385	0.7864	0.9155	0.9794	0.4569	na	—	0.8237	0.0082	*<0.001
CSL	0.9475	0.9703	0.9824	1.000	0.6576	na	0.9849	—	0.0012	*<0.001
NAY	0.9656	0.9984	0.8418	0.4965	0.4101	na	0.4206	0.5707	—	*<0.001
GC	0.9458	0.9637	0.9987	0.9977	0.9743	na	0.9715	0.9874	0.6518	—

Table 3

Estimates of population subdivision θ (Weir and Cockerham, 1984) for allozymes of *Thunnus albacares* partitioned into longitudinal regions θ_L (regions) (i.e. coastal-intermediate-offshore) and samples θ_0 (overall). n.v. = negative values. P = probability of significance of subdivision estimations. Significance of single-locus values was corrected with an initial level of 0.006 (0.05/8 loci). Means and standard error were obtained by the jackknife method. Confidence intervals obtained by 1000 resamplings through bootstrapping are also shown. * = signifcant values after Bonferroni correction.

Locus	$\theta_{L\text{ (regions)}}$	P	$\theta_{0\text{(overall)}}$	P
*Aat-2**	0.0152	0.072	0.029	0.007
Glud	0.0009	0.227	0.086	>0.006*
*Gpi-1**	0.0058	0.086	0.003	0.210
*Gpi-2**	0.0014	0.780	0.0037	0.007
La	n.v.	0.012	0.13	>0.006*
Lgg	0.0073	0.004	0.27	>0.006
*Pap-1**	n.v.	0.068	0.024	0.001
6-Pgd	0.044	>0.006*	0.02	>0.006*
Mean	0.0067 ±0.0042		0.048 ±0.022	
CI 95%	0.0003–0.0199		0.019–0.101	

Table 4

Pairwise-regions comparison of allele frequencies for *Thunnus albacares*. Probabilities of nonheterogeneity for allozymes (based on exact tests) are above the diagonal (—) and RAPDs are below the diagonal (—). * = significant after corrected for multiple tests (Rice, 1989).

Region	Coastal	Intermediate	Offshore
Coastal	—	0.0632	0.0043*
Intermediate	0.9998	—	0.5384
Offshore	0.9039	0.9126	—

were polymorphic for all samples (Table 1). No significant heterogeneity of RAPD allele frequencies was found for any locus between any paired sample comparison, among all collections, nor among latitudinal or longitudinal regions (P=0.4806).

The mean θ value for all fragments and samples (overall), as well as regional estimations derived from RAPDs (0.0302), were not significantly different from zero and displayed some negative values. Estimations of gene flow between sample pairs (M_θ) from RAPD data averaged 29.2 migrants per generation. The evaluation of the relationships between geographic distances and the gene flow estimations in pairwise collections (M_θ), through the Mantel test, showed a nonsignificant correlation (r^2=0.413, P=0.984).

Discussion

The test of conformance to the Hardy-Weinberg frequencies showed significant differences in *Lgg* and *Pap-F** loci only in the Gulf of California sample, where polymorphism at those loci was also consistently found. Similar results were obtained, with smaller differences in locus *Aat-S** from the west of the Revillagigedo Islands and the Gulf of California samples. Considering the fact that our samples were provided by the commercial fleet, they could have included representatives of different schools with differences in genotypic distributions originated by differences in age classes or sexual ratios (or for both) among schools because recruitment of individuals into new schools has been reported to be mainly by aggregating individuals of

Table 5

Comparison of *Gpi-F** allele frequencies for *Thunnus albacares* among data from the present study and those reported in Ward et al. (1994). — = data absent.

Locus Allele	Western/Central[1]	Eastern[1]	Eastern (present data) Offshore	Intermediate	Coastal	Pooled
*Gpi-F**						
135	0.026	0.100	0.103	0.147	0.163	0.145
100	0.640	0.269	0.301	0.412	0.301	0.307
75	0.332	0.631	0.596	0.441	0.566	0.548
40	0.002	—	—	—	—	—
n	346	178	83	17	196	296

[1] Allele frequencies for *Gpi-F** reported in Ward et al. (1994).

similar sizes (Collette and Nauen, 1983). Because recruitment to the original tuna schools has been reported as well (Kimley and Holloway, 1999), random processes could also induce differences in genotypic frequencies that favor aggregation of some genotypes, while segregating some others, causing a kind of Wahlund effect that is reflected by a heterozygous deficit as shown by the homozygous excess for loci and locations having HW deviations, especially as shown in the Gulf of California sample.

The estimations of population structure based on allozymes showed a small but significant value different from zero (θ=0.048; $P<0.01$). The Gulf of California sample contributed to the significant subdivision value as shown when that collection was excluded from the regional subdivision analysis, as well as to significant heterogeneity of its allele frequencies when paired comparisons were made.

The small value of θ for overall estimations on RAPD data is probably due to the small sample size. The negative values of θ from overall and regional estimations resulted from subtracting the large value of the correction derived from the variation expected of the sample sizes from the small value of variation due to fluctuations in allele frequencies. The fact that RAPD data are considered dominant could reduce information about the true allele distributions by subestimation of null allele frequencies notwithstanding the correction applied to recessive genotypes, which is dependent on the sample sizes (Lynch and Milligan, 1994). Other assumptions for RAPD data limit the value of this marker, especially when estimations are derived from a small number of loci and sample sizes. Additional constraints are related to the limited number of alleles (two) to estimate dominant markers, which tend to subestimate the polymorphism and thus reduce the significance of relatively small discrepancies in allele distributions.

No differentiation between coastal and offshore samples was found in our study because of the slight, nonsignificant differences in the estimation of the subdivision by regions. Although the overall estimation was not different from zero, the allele homogeneity analysis showed allele-frequency heterogeneity between coastal and offshore samples, and nonheterogeneity between coastal and intermediate samples.

These results are consistent with the migration reports through tagging studies; evidence exists for the presence of two main yellowfin tuna groups in the eastern Pacific that mix to some extent (Fink and Bayliff, 1970) and that migrate longshore from around the 20°N to the mouth of the Gulf of California and to the zone between the Revillagigedo and the Clipperton islands, and back again (Joseph et al., 1964; Fink and Bayliff, 1970), although longitudinal movements are restricted to the limits of yellowfin regulatory area (CYRA). Similarly, important northward movements along the coasts to the mouth of the Gulf of California, and subsequently to the western coasts of Baja California, have been reported. Although the estimation of θ for allozymes showed a significant value, it was notably influenced by the heterogeneity found between the Gulf of California sample and all other samples.

Discarding the variation displayed by loci *La*, *Lgg*, and *Pap-F**, originating mainly from Gulf of California sample, the estimation of subdivision was still marginally significant after Bonferroni correction, which should be considered as evidence that the Gulf of California sample may represent a partially isolated population with different allele frequencies. Oceanographic conditions inside the Gulf are somewhat different from those of the Pacific Ocean where there are warmer waters at the end of the year, especially during yellowfin tuna spawning seasons. There is also high productivity characterized by the presence of significant biomass abundance of sardine or anchovy schools (Cisneros-Mata et al., 1995), which represents opportunities to establish the feeding and consequently the spawning grounds for eastern Pacific yellowfin tuna. Likewise, there is a trend of migratory movements through the Gulf of California by different groups of yellowfin tunas (Fink and Bayliff, 1970). These movements promote stock mixing and help to explain the wide polymorphism displayed in this sample, in contrast to the weak variation found in other samples from the coast and offshore regions. Further genetic research, including sequential temporal sampling of young fishes in order to ensure the presence of individuals that originated in discrete spawning grounds, should be undertaken to prove the presence of an independent unit inside the Gulf of California, which, if confirmed, might necessitate new stock management strategies.

Allele frequencies for *Gpi-F** locus found in the present study, apparently, correspond to those reported by Ward et al. (1994 and 1997). These authors reported a higher proportion of the allele *Gpi-F*75* (0.571) in the eastern Pacific region and a gradual decrement of the same allele toward the central (0.423) and western Pacific regions (0.330), where allele *Gpi-F*100* (0.650) had the higher proportion. In the present study, the highest frequencies for the allele *Gpi-F*75* corresponded to the region of the eastern Pacific, situated in the limits of the yellowfin tuna regulatory area (offshore region), and there was a slight decrease in frequencies towards the coastal area (Table 5). Furthermore, allele frequencies for the *Gpi-F*75* allele from the coastal locality, Colima (0.444), and the intermediate locality southeast, Clipperton Islands (0.409), have coincidences with those reported by Ward et al. (1994) for the collection Hawaii 92 (0.423) in the central Pacific region.

The similarities in the *Gpi-F** allelic frequencies between eastern (Colima and Clipperton) and central Pacific samples (Hawaii 92) might possibly be attributed to the extended migrations of yellowfin tuna in the eastern Pacific brought about by the strong influence of warm waters on tuna movements because of the increased depth of the thermocline layer in that area, which was reflected by a decrease in catches (Joseph and Miller, 1988; Wild, 1994) and which possibly led to the mixing of the eastern and central Pacific stocks.

The low number of RAPD loci analyzed and the uncertainty of fulfilling some assumptions, such as the genetic identity of each band needed for qualitative and quantitative interpretation of data in terms of allelic frequencies, do not allow us to consider our estimations of subdivision reliable with the RAPD method. Additionally, the lack of reliability of estimations associated with high sampling variances by using randomly collected fishery samples highlights the need to design more efficient spatial and temporal sampling strategies in local and wide areas, as well as the need for alternative hypervariable markers to assess the divergence patterns observed in highly migratory species.

Acknowledgments

We are grateful to Ernesto Escobar from Pescados Industrializados S. A. PINSA for allowing the sampling, and Robert Olson from IATTC for providing the Gulf of California samples. We thank Monica Domínguez-Lopez, Yolanda Hornelas-Orozco, Evangelina Castillo, and Alma Hernández-Pérez, for collection and processing of samples, Luis Eguiarte and Valeria Souza for the facilities provided in their laboratory and three anonymous reviewers for their valuable comments. This manuscript benefited from the critical reading of John Graves, Jan McDowell, and Barbara Rutan. Funding for this project was provided by the Programa de Apoyo a Estudiantes de Posgrado (PADEP) and by the project IN20598 of the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica. Dirección General de Asuntos del Personal Académico, Universidad Nacional Autónoma de México.

Literature cited

Barret, I., and H. Tsuyuki.
1967. Serum transferrin polymorphism in some scombroid fishes. Copeia 1967:551–557.

Bielawski, J. P. and D. E. Pumo.
1997. Randomly amplified polymorphic DNA (RAPD) analysis of Atlantic coast striped bass. Heredity 78:32–40.

Cisneros-Mata, M. A., Nevarez-Martinez, M. O., and Hammann, M. G.
1995. The rise and fall of the Pacific sardine, *Sardinops sagax caeruleus* Girard, in the Gulf of California, Mexico. CALCOFI Rep. 36:136–143.

Collette, B. B., and C. E. Nauen.
1983. Scombrids of the world: an annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. *In* FAO species catalogue, vol. 2, 137 p. FAO Fish. Synop. 1325. FAO, Rome.

Fink, B. D., and W. H. Bayliff.
1970. Migrations of yellowfin and skipjack tuna in the eastern Pacific Ocean as determined by tagging experiments, 1952–1964. Inter-Am. Trop. Tuna Comm. Bull. 15:1–227.

Fujino, K.
1970. Immunological and biochemical genetics of tunas. Trans. Am. Fish. Soc. 99:152–178.

Gibbs, R. H., and B. B. Collette.
1967. Comparative anatomy and systematics of the tunas, genus *Thunnus*. Fish. Bull. 86:835–838.

Godsil, H. C., and E. C. Greenhood.
1951. A comparison of the populations of yellowfin tuna (*Neothunnus macropterus*) from the eastern and central Pacific, 33 p. Calif. Dep. Fish Game Fish. Bull. 82.

Harris, H., and D. A. Hopkinson.
1976. Handbook of enzyme electrophoresis in human genetics, 273 p. Am. Elsevier Publishing Co., New York, NY.

Hellberg, M. E.
1994. Relationships between inferred levels of gene flow and geographic distance in a philopatric coral, *Ballanophyllia elegans*. Evolution 48:1829–1854.

IATTC (Inter-American Tropical Tuna Commission).
1975. Annual report of the Inter-American Tropical Tuna Commission, 169 p. IATTC, LaJolla, CA.
1999. Annual report of the Inter-American Tropical Tuna Commission, 357 p. IATTC, LaJolla, CA.

Joseph, J., F. G. Alverson, B. D. Fink, and E. B. Davidoff.
1964. A review of the population structure of yellowfin tuna, *Thunnus albacares*, in the eastern Pacific Ocean. Inter-Am. Trop. Tuna Comm. Bull. 9(2):53–112.

Joseph, J., and F. R. Miller.
1988. El Niño and the surface fishery for tunas in the eastern Pacific. Proceedings of the Tuna Fisheries Research Conference, Japan Fish. Far Seas Fish. Res. Lab. Maguro Gyogyo Kyogikai Gijiroku, Suisancho-Enyo Suisan Kenkyusho:199–207

Kenji, S.
1998. Genetic variation and local differentiation in the Pacific cod *Gadus macrocephalus* around Japan revealed by mtDNA and RAPD markers. Fish. Sci. 64(5):673–679.

Kimley, A. P., and C. F. Holloway.
1999. School fidelity and homing synchronicity of yellowfin tuna, *Thunnus albacares*. Mar. Biol. 133:307–317.

Kurogane, K., and Y. Hiyama.
1957. Morphometric comparison of the yellowfin tuna taken from the Equatorial Pacific. Jpn. Soc. Sci. Fish. Bull. 23: 388–293.

Leis, J. M., T. Trnski, M. Harmelin-Vivien, J.-P. Renon, V. Dufour, M. K. El Moundi, and R. Galzin.
1991. High concentrations of tuna larvae (Pisces: Scombridae) in near-reef waters of french polynesia (Society and Taumotu islands). Bull. Mar. Sci. 48(1):150–158.

Le Corre, S., S. Dumolin-Lapegue, and A. Kremer.
1997. Genetic variation at allozyme and RAPD loci in sessile oak *Quercus petraea* (Matt.) Liebl.: the role of history and geography. Mol. Ecol. 6:519–529.

Lynch, M., and B. G. Milligan.
1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3:91–99.

Mamuris, Z., A. P. Apostodilis, A. J. Theodoru, and C. Triantaphyllidis.
1998. Application of random amplified polymorphic DNA (RAPD) markers to evaluate intraspecific genetic variation in red mullet (*Mullus barbatus*). Mar. Biol. 132: 171–178.

Raymond, M. L., and F. Rousset.
1995. An exact test for population differentiation. Evolution 49:1280–1283.

Rice, W. R.
1989. Analyzing tables of statistical tests. Evolution 43: 223–225.

Sambrook, J., E. F. Fritsch, and T. Maniatis.
1989. Molecular cloning: a laboratory manual, 2nd ed., 999 p. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Scoles, D. R., and J. E. Graves.
1993. Genetic analysis of the population structure of yellowfin tuna, *Thunnus albacares*, from the Pacific Ocean. Fish. Bull. 91:690–698.

Schaefer, K. M.
1991. Geographical variation in morphometric characters and gill-raker counts of yellowfin tuna *Thunnus albacares*, from the Pacific Ocean. Fish. Bull. 9:690–698.

Schaefer, M. B.
1955. Morphometric comparison of yellowfin tuna from southeast Polynesia, Central America, and Hawaii. Inter-Am. Trop. Tuna Comm. Bull. 1:89–136.

Sharp, G. D.
1978. Behavioral and physiological properties of tunas and their effects on vulnerability to fishing gear. *In* The physiological ecology of tunas (G. D. Sharp and A. E. Dizon, eds.), p. 397–449. Academic Press, New York, NY.

Slatkin, M.
1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279.

Sokal, R., and F. J. Rohlf.
1995. Biometry, 3rd ed., 776 p. W. H. Freeman and Co., New York, NY.

Suzuki, Z., P. K. Tomlinson, and M. Honwa.
1978. Population structure of Pacific yellowfin tuna. Inter-Am. Trop. Tuna Comm. Bull. 17:273–441.

Ward, R. D., N. G. Elliott, P. M. Grewe, and A. Smolenski.
1994. Allozyme and mitochondrial DNA variation in yellowfin tuna (*Thunnus albacares*) from the Pacific Ocean. Mar. Biol. 118:531–539.

Ward, R. D., N. G. Elliott, B. H. Innes, A. J. Smolenski, and P. M. Grewe.
1997. Global population structure of yellowfin tuna, *Thunnus albacares*, inferred from allozyme and mitochondrial DNA variation. Fish. Bull. 95:566–575.

Weir, B. S.
1990. Genetic data analysis, 377 p. Sinauer, Sunderland, MA.

Weir, B. S., and C. C. Cockerham.
1984. Estimating F statistics for the analysis of population structure. Evolution 38:1358–1370.

Welsh, J., C. Petersen, and M. McCelland.
1991. Polymorphisms generated by arbitrary primed PCR in the mouse: application to strain identification and genetic mapping. Nucl. Acids Res. 19:303–306.

Wild, A.
1994. A review of the biology and fisheries for yellowfin tuna, *T. albacares*, in the eastern Pacific Ocean. FAO Fish. Tech. Pap. 336(2):52–107.

Williams, J. G, A. R. Kubelik, J. Livak, J. A. Rafalaski, and S. V. Tingey.
1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18: 6531–6535.

The early life history of swordfish (*Xiphias gladius*) in the western North Atlantic

John Jeffrey Govoni
Elisabeth H. Laban
Jonathan A. Hare
Center for Coastal Fisheries and Habitat Research
National Oceanic and Atmospheric Administration
101 Pivers Island Road
Beaufort, North Carolina 28516-9722
E-mail address (for J. J. Govoni): Jeff.Govoni@noaa.gov

Abstract—Lengths and ages of swordfish (*Xiphias gladius*) estimated from increments on otoliths of larvae collected in the Caribbean Sea, Florida Straits, and off the southeastern United States, indicated two growth phases. Larvae complete yolk and oil globule absorption 5 to 6 days after hatching (DAH). Larvae <13 mm preserved standard length (PSL) grow slowly (~0.3 mm/d); larvae from 13 to 115 mm PSL grow rapidly (~6 mm/d). The acceleration in growth rate at 13 days follows an abrupt (within 3 days) change in diet, and in jaw and alimentary canal structure. The diet of swordfish larvae is limited. Larvae <8 mm PSL from the Caribbean, Gulf of Mexico, and off the southeastern United States eat exclusively copepods, primarily of one genus, *Corycaeus*. Larvae 9 to 11 mm eat copepods and chaetognaths; larvae >11 mm eat exclusively neustonic fish larvae. This diet indicates that young larvae <11 mm occupy the near-surface pelagia, whereas, older and longer larvae are neustonic. Spawning dates for larvae collected in various regions of the western North Atlantic, along with the abundance and spatial distribution of the youngest larvae, indicate that spawning peaks in three seasons and in five regions. Swordfish spawn in the Caribbean Sea, or possibly to the east, in winter, and in the western Gulf of Mexico in spring. Elsewhere swordfish spawn year-round, but spawning peaks in the spring in the north-central Gulf of Mexico, in the summer off southern Florida, and in the spring and early summer off the southeastern United States. The western Gulf Stream frontal zone is the focus of spawning off the southeastern coast of the United States, whereas spawning in the Gulf of Mexico seems to be focused in the vicinity of the Gulf Loop Current. Larvae may use the Gulf of Mexico and the outer continental shelf off the east coast of the United States as nursery areas. Some larvae may be transported northward, but trans-Atlantic transport of larvae is unlikely.

Manuscript approved for publication 17 April 2003 by Scientific Editor.

Manuscript received 26 June 2003 at NMFS Scientific Publications Office.

Fish. Bull. 101:778–789 (2003).

Swordfish (*Xiphias gladius*) live in warm waters of the world's oceans, as well as in large enclosed basins such as the Caribbean and Mediterranean seas, and the Gulf of Mexico (Berkeley, 1983). Swordfish are highly migratory throughout their global range. The worldwide population structure, as currently understood, has at least three breeding units: Mediterranean, northwestern Atlantic to the tropical South Atlantic, and Indo-Pacific (Kotoulas et al., 1995; Chow and Takeyama, 2000; Reeb et al., 2000). For the purpose of fishery management, the International Commission for the Conservation of Atlantic Tunas (ICCAT) recognizes only North Atlantic and South Atlantic stocks. Possible genetic exchange between eastern and western North Atlantic populations is incompletely documented.

Swordfish reportedly spawn year-round in the western North Atlantic in different seasons and regions. Spawning season and location has been inferred from the abundance of small larvae (Gorbunova, 1969; Richards and Potthoff, 1980; Potthoff and Kelley, 1982; Grall et al., 1983; Govoni et al., 2000), gonad maturation (LaMonte, 1944; Beckett, 1975), or oocyte cytology (Taylor and Murphy, 1992; Arocha, 1997; Arocha, 2002). The observation of live females with running eggs, hooked on long-lines, and followed to the fishing vessel by several smaller males (Lee[1]; Berkeley[2]) corroborates spawning in some seasons and locations. Although gonad condition and oocyte status can indicate spawning season, the resolution of spawning location can be ambiguous with these methods because mature gonads and hydrated oocytes can be found in several seasons within the range of these highly migratory fishes. The determination of age and the distribution of young larvae, along with realistic estimates of water velocity and trajectory, help to resolve this ambiguity.

Beyond spawning, the early life history of swordfish in any ocean is incompletely described (Palko et al., 1981). Larvae undergo a stark change in physical appearance between ~8 and 13 mm preserved standard length (PSL), from a typical scombroid larval form to a juvenile istiophorid one (Collette et al., 1984). At this juncture in development, larvae develop characteristic preorbital, supraorbital, posttemporal, and preopercular spines; enlarged and spinous dorsal, ventral, and lateral scale anlagen; and a continuous long dorsal fin that extends along most of the dorsal aspect. Swordfish retain these larval characters until they are at least 188 mm PSL (Arata, 1954; Potthoff and Kelley, 1982), a size at which most fishes are considered juveniles. By using the ages and lengths of larvae hatched in the laboratory and reared through yolk and oil globule absorption (Sanzo, 1910; Yasuda et al., 1978), along with length frequencies of larvae caught in the western North Atlantic,

---

[1] Lee, D. J. 1995. Personal commun. Southeast Fisheries Science Center, NMFS, 75 Virginia Beach Drive, Miami, FL 33149.

[2] Berkeley, S. A. 1998. Personal commun. Long Marine Laboratory, Univ. California, Santa Cruz, 100 Shaffer Road, Santa Cruz, CA 95060.

Arata (1954) inferred the age and growth of larvae from 6 to 192 mm preserved total length (PTT). Aside from this effort, the age of larval swordfish has been undetermined and growth has not been described. Diets of larvae have been reported (Gorbunova, 1969), but the apparent transition in diet has neither been detailed nor reconciled with changes in physical features and growth. Similarly, the vertical distribution of larvae has not been reconciled with their diet or growth. Most larvae are collected near the surface of the ocean, typically in neuston nets, but some larvae are collected in nets that sample below the surface (Govoni et al., 2000).

In the present study, we resolve and summarize the early life history of swordfish in the western North Atlantic. We report estimated age, describe growth, relate growth to feeding, morphological features, and vertical distribution, and infer spawning time and location and the sources and fates of swordfish larvae. This study supplements that of Govoni et al. (2000) by providing the dimension of time, i.e. age of larvae, to the spatial distribution and possible transport of these larvae.

Methods

Collections of larvae

Ichthyoplankton collections from cruises in 1989 (in the northeastern Caribbean about the Lesser Antilles), 1991 and 1997 (off the southeastern United States), and 2000 (in the Straits of Florida and off the southeastern United States), produced 63 larvae that were preserved in 95% ethanol (for examination of otolith microstructure). Samples were collected either from the neuston (i.e. taken with a 1.0×0.5 m neuston net) or from depth intervals (i.e. taken with a 1-m MOCNESS [multiple opening and closing net and environmental sampling system] [Wiebe et al., 1976]). Larvae were measured for preserved standard length (PSL), the conventional length measure for larval fishes (Kendall et al., 1984), and lower-jaw-fork-length (LJFL), the measure in common use for juvenile and adult swordfish (Megalofonou et al., 1995).

Otolith excision and examination

Of the 63 larvae collected, sagittae were found and successfully excised from 37 larvae, lapilli from 32, and asterisci from six. Otoliths were mounted on glass slides and dried before examination. Broken sagittae and lapilli, and some large sagittae, were embedded in plastic, sectioned with a saw, and polished (Secor et al., 1991).

Otolith growth increments were counted along the longest axis of each sagitta and lapillus by using a compound light-transmission microscope; increments in asterisci were fewer and less defined and were not counted. Three blind counts were made by the same observer. Although increments were consistently visible on both sagittae and lapilli, counts from individual larvae were greater on sagittae (Student's *t*-test; $P<0.001$). Standardized counts (the standard deviate of each repeated count) on the right and left sagittae were not significantly different (nested ANOVA; $P<0.05$). Increment counts from either the left or right sagitta, decided by coin toss, were used for age and growth rate determination. The mean of three replicate counts was rounded to a whole number.

Increment counts from sagittae were used to estimate larval age. Increments were assumed to form daily (Campana, 2001). The core increment was assumed to form at hatching (Jones, 1986). The first increment outside of the core increment was counted as one. Age from hatching (AFH) was the number of increments counted from the core increment on sagittae. This definition differs from that of Prince et al. (1991) who counted the core increment as increment one for the istiophorid blue marlin (*Makaira nigricans)* and for a single larval swordfish that was 8.5 PSL. The radius of each otolith was measured by image analysis.

Growth model

The best empirical fit among a suite of regressions of estimated age (AFH) and length (PSL)—linear, polynomial, and piece-wise, and moving—was chosen to describe somatic growth (Forbes and Lopez, 1989; Hare and Cowen, 1995; Rogers et al., 2001). Criteria for best fit were the following: the interpretation of fit from graphical display; regression coefficients (r^2), and dispersion or convergence of regression residuals. The model of best fit was also applied to the estimated age and lower jaw fork length (LJFL) to allow comparison with published accounts of juvenile swordfish growth.

Diet

Sixty-eight specimens from the present collections and from Govoni et al. (2000), all with undamaged alimentary canals, were examined for gut contents. These specimens were taken in the northeastern Caribbean (3 specimens), Gulf of Mexico (5), and off the southeastern coast of the United States (60). Food was identified to the lowest taxon possible following Govoni et al. (1983).

Physical features of larvae

Histological sections of three larvae, 21.5, 30.0, and 52.0 mm PSL, were cut as a preliminary aid to the location of otoliths within the cranium and to determine the histological constitution of the larval alimentary canal.

Time and location of spawning

Spawning dates were estimated from the ages of larvae (estimated from the growth model (AFH)), plus 3 days (the incubation period at 25°C for swordfish eggs given by Yasuda et al. [1978]). Spawning dates are thus days from fertilization (DFF). Spawning location was inferred by applying DFF to larval swordfish lengths reported in the present study, as well as lengths given in Govoni et al. (2000), taking into consideration the time that eggs and larvae were at large and adrift (DFF) and the location where

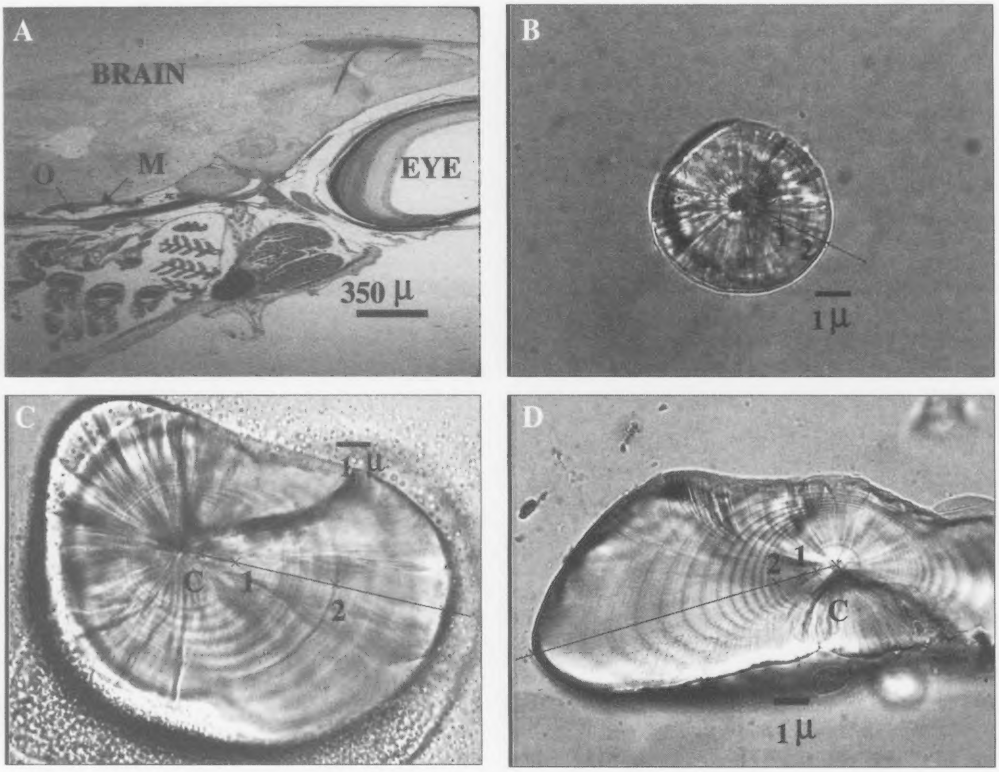


Figure 1

Otoliths of larval swordfish (*Xiphias gladius*): (**A**) histological section through the braincase of a 21.0-mm larva; (**B**) left sagitta from a 5.4-mm-PSL larva; (**C**) histological section of a right sagitta of a 33.3-mm-PSL larva; and (**D**) transverse section of a right sagitta of a 47.5-mm-PSL larva (C=core increment; 1=first check; 2=second check; M=macula within the otolith vestibule; O=otolith; x's (B–D) mark intervals of measurement).

larvae were collected, and back-calculating the geographic origin of eggs with mean axial trajectories and velocities of water currents for the Yucatan, Gulf Loop, Florida Currents, and the Gulf Stream (~1.5 m/s [Maul and Vukovich, 1993; Olson et al., 1994; Boicourt et al., 1998]) and the Caribbean Sea (~0.2 m/s [Mooers and Maul, 1998]).

Results

Otolith structure and increment counts

Sagittae and lapilli were round, extremely small, and lacked rostra or sulci in larvae <5 mm PSL (Fig. 1, A and B). A rostrum developed on sagittae at ~5.5 mm PSL (Fig. 1C). Lapilli did not develop rostra, and remained symmetrical with growth.

Two checks, distinct zones of irregular increment spacing and opacity, were evident on most sagittae (Fig. 1, B–D). The first check was evident at the third increment on all sagittae examined. The second check was found on sagittae from larvae >3.8 PSL but varied from the seventh to tenth increment.

Growth model

A piece-wise regression (Table 1; Equations 5–7) with two linear segments provided the best fit with biologically realistic parameters. An assigned intercept of 3.2 mm PSL was used for the first segment; this value was obtained by adjusting the length at hatching with the scale given by Yasuda et al. (1978) and by accounting for shrinkage due to preservation. Growth rate for the first segment was 0.3 mm/d and 5.9 mm/d PSL for the second segment (Fig. 2A). The intersection of the two linear segments was at an estimated age of 13.3 d AFH., 3 to 6 d after the observed second check. The PSL of larvae at the intersection was 11.0 mm.

Growth rate in LJFL, also modeled with a piecewise regression, was 0.2 mm/d for the first segment (the upper and lower jaws of larvae <11 mm PSL are of equal length,

Table 1

Summary of models evaluated for describing growth of larval swordfish. Models 1–6 were preserved standard length (PSL) as a function of estimated age from hatching (AFH); model 7 was lower jaw fork length (LJFL) as a function of AFH. There were three model types: linear regression (models 1 and 2), 2nd order polynomial (models 3 and 4), and piecewise regression (models 5–7). Y-intercepts (model parameter a) were estimated by the regression in Equations 1, 3, and 5 but were fixed at an observed length at hatching of 3.2 mm from Yasuda et al. (1978) in Equations 2, 4, 6, and 7 ("ns" denotes that a was not significantly different from an assigned a of 3.2 at α=0.05; * = y-intercept significantly different from 3.2 at α=0.05; na = not applicable; b and c are slopes; and d is the inflection point).

	Model parameters				Model fit	
Model	a	b	c	d	r^2	Residuals
1. $PSL = a + b\text{AFH}$	–39.11*	4.15	na	na	0.81	not normal
2. $PSL = 3.2 + b\text{AFH}$	3.20 na	1.47	na	na	0.81	not normal
3. $PSL = a + b\text{AFH} + c\text{AFH}^2$	11.31 ns	–2.77	0.22	na	0.92	normal
4. $PSL = 3.2 + b\text{AFH} + c\text{AFH}^2$	3.20 na	–1.75	0.19	na	0.91	normal
5. $PSL = a + b\text{AFH} + c(\text{AFH} - d) \times (\text{AFH}\ \ d)$	–2.22*	0.76	5.10	13.53	0.89	normal
6. $PSL = 3.2 + b\text{AFH} + c\,(\text{AFH} - d) \times (\text{AFH} \geq d)$	3.20 na	0.26	5.60	13.29	0.89	normal
7. $LJFL = 3.2 + b\text{AFH} + c\,(\text{AFH} - d) \times (\text{AFH} \geq d)$	3.20 na	0.16	3.28	11.24	0.77	ns

Table 2

Diet composition of 68 larval swordfish (*Xiphias gladius*) in the western North Atlantic.

Diet item	% Frequency of occurrence (among larvae with food)	% total number (among all food items)
Copepodites and adult copepods (unidentifiable)	5	2
calanoids		
Eucalanus	2	1
cyclopoids		
Corycaeus spp.	59	74
Oithona spp.	4	2
Chaetognaths	2	1
Larval and juvenile fishes	33	16
Invertebrate eggs	2	2
Chyme	5	2

hence PSL=LJFL) and 3.4 mm/d for the second segment (Fig. 2B).

The fit of the piecewise regression for PSL and LJFL was unchanged by inclusion or exclusion of the estimated age of the largest larva.

Diet

The diet of larvae is limited and transitional. Larvae <8.3 mm PSL ate copepods exclusively, primarily a single cyclopoid genus, *Corycaeus* spp., but also another cyclopoid, *Oithona* spp., and the calanoid *Eucalanus* (Table 2). Larvae 9.0 to 11.0 mm PSL ate copepods (Fig. 3A) and chaeto-

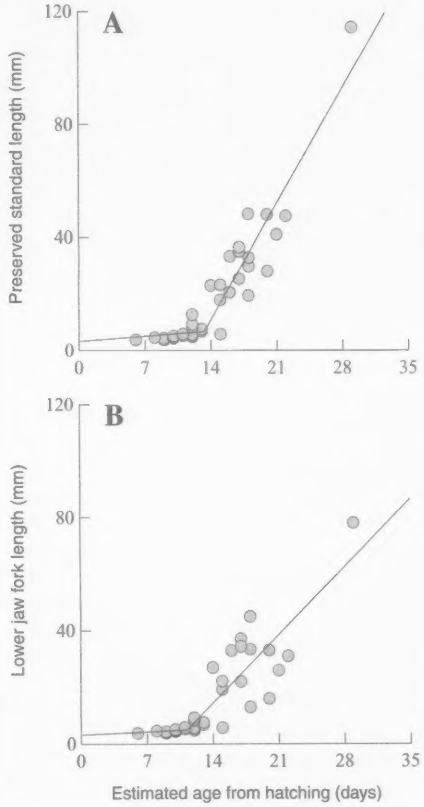


Figure 2

Growth of larval swordfish from the western North Atlantic: (**A**) estimated age and PSL; (**B**) estimated age and LJFL.

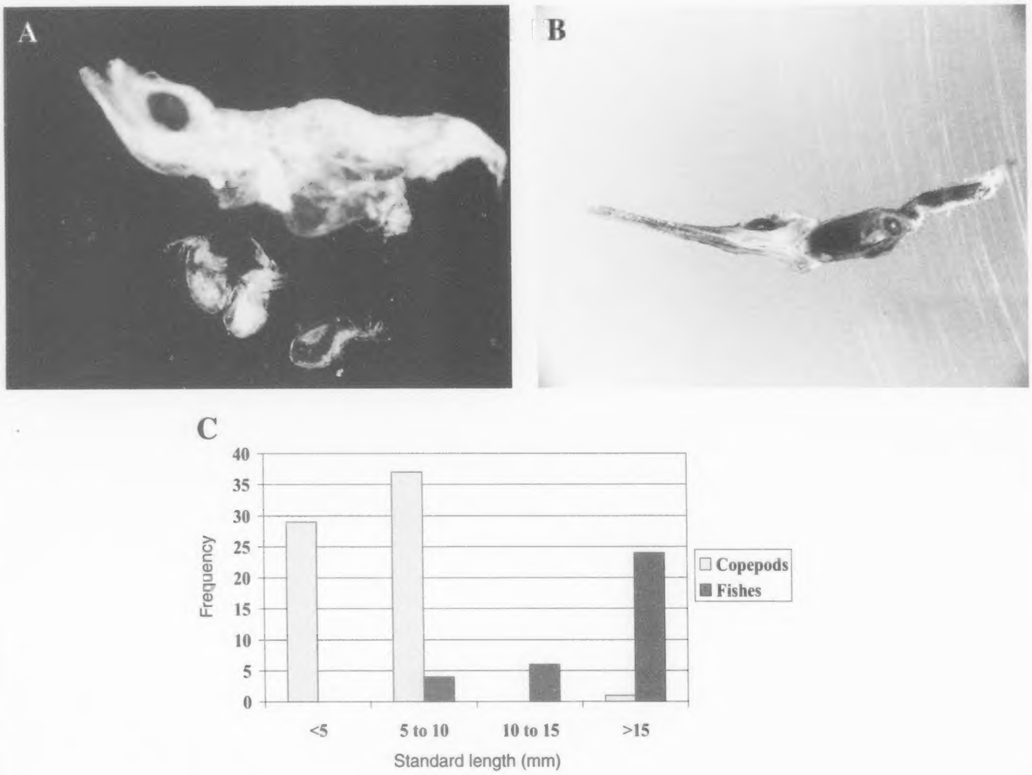


Figure 3

Diet composition of larval swordfish from the western North Atlantic: (**A**) a 3.9-mm-PSL larva and cyclopoid copepods (genus *Corycaeus*) that were removed from the gut; (**B**) a 12.7-mm larva with a larval fish in its gut; and (**C**) the frequency of occurrence of copepods and larval fishes in the guts of larval swordfish.

gnaths. Larvae >11.0 mm PSL ate almost exclusively larval and juvenile fishes (Fig. 3, B and C). Remnant jaws and heavy pigmentation of many of the fishes eaten, indicated that most were neustonic. One exocoetid was identified by intact pectoral fins and counts of vertebrae.

Jaw and alimentary canal structure

The structure of the alimentary canal and jaws changed concomitantly. The alimentary canal began to change from three segments (foregut, midgut and hindgut), typical of larval fishes (Govoni et al., 1986a), to four segments (esophagus, stomach, anterior intestine, and posterior intestine) between 9.0 and 12.0 mm PSL. Jaws change during this period from the beak-like jaws to the elongate rostral bill of the istiophorids (Fig. 3, A and B). Gastric glands were evident in the fundic region of the stomach (ventricili-gastric cecum), close to the junction with the esophagus in the 30.0-mm-PSL larva (Fig. 4, A and B). The pyloric region of the stomach (pars pylorica) was evident in the 21.5-mm-PSL larva and the 30.0-mm larva.

Time and location of spawning

Back-calculated spawning dates demonstrated year-round spawning and peaks in three seasons and five regions (Fig. 5, A–C). Larvae collected in the eastern Caribbean were spawned in the winter (northern hemisphere) only. Larvae collected in the western Gulf of Mexico were spawned in spring. In the north-central Gulf of Mexico, larvae were spawned in all seasons, but spawning peaked in spring. Off south Florida, larvae were spawned in all seasons, and spawning peaked peaked in spring. Larvae collected off the southeastern United States were spawned throughout the year whereas larvae collected in the north-central Gulf of Mexico and in southern Florida waters were spawned mostly in spring and early summer.

Modes of the number of larvae collected and their estimated DFF advanced slightly in day of the year from the north-central Gulf of Mexico to off the southeastern United States. Larvae <10 DFF were collected both in the north-central Gulf of Mexico and off the southeastern United States (Fig. 5D), but not off South Florida.

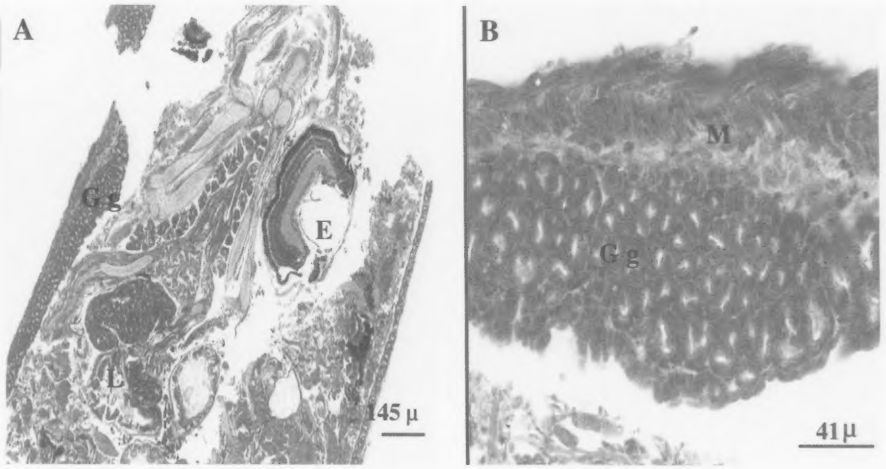


Figure 4

Photomicrographs of a 30.0-mm-PSL swordfish larva: (**A**) fundic stomach; (**B**) gastric glands in stomach mucosa (Gg=gastric glands; M=mucosa; L=stomach lumen with larval fish remains; E=eye of eaten larval fish).

Discussion

The first check on sagittae apparently corresponds with the completion of yolk and oil globule absorption and the beginning of feeding. Sanzo (1910) and Yasuda et al. (1978) reported hatching 3 days after fertilization when larvae were ~4 mm live total length (LTL), or 3.8 mm live standard length (scaled from their drawings), and complete yolk and oil globule absorption 8 d after fertilization (or 5 DAH) when larvae were ~5 mm LTL, or 4.3 mm live standard length. Larvae from the present material had completed yolk and oil absorption between 3.8 and 3.9 mm PSL and had the first check 3 increments after the core increment. Temperature and feeding influence the growth rate of fish larvae and their otoliths, but larvae are typically collected in water 25 ±1°C (Arata, 1954; Tåning, 1955; Tibbo and Lauzier, 1969; Markle, 1975; Govoni et al., 2000), as were the larvae collected for age determination. This temperature is common to the Gulf Stream and its progenitor currents (Schmitz et al., 1993; Hitchcock et al., 1994), and is similar to the temperature used to rear larvae (Sanzo, 1910; Yasuda et al., 1978). The difference in length at complete yolk and oil absorption between Sanzo (1910) and Yasuda et al. (1978) and the present collections probably owes to shrinkage of larvae with death and preservation (e.g. Theilacker, 1980).

The second check follows concomitant changes in diet and morphological features that take place between 8 and 13 mm PSL or from 7 to 11 DFH. An acceleration in somatic growth follows the second check within a day or so. Young swordfish larvae eat copepods; older larvae other larval fishes. The most striking morphological change of larval swordfish is in the jaws. Swordfish larvae <13 mm SL have beak-like jaws that are typical of the larval scombroid fishes (Collette et al., 1984), particularly those of the wahoo (*Acanthocybium solandri*) and scaleless tuna (*Gymnosarda unicolor*); older larvae develop bill-like jaws with elongate rostral cartilages anterior of the premaxillaries and equally elongate mandibles (McGowan, 1988). The constitution of the alimentary canal changes as well. The development of a functional stomach with gastric glands in larval swordfish, which typically arises during the metamorphosis of fishes (Govoni et al., 1986a), is evident in the larvae of other scombroid fishes where it is accompanied similarly by a change in diet from zooplankton to fish (Kaji et al., 1999; Shoji et al., 1999). A switch from zooplankton to fish is common among istiophorid larvae, but it is neither as exclusive nor abrupt (Voss, 1953; Lipskaya and Gorbunova, 1977) as it is with swordfish. Accelerated growth after such a dietary shift is also a common trait of scombroid larvae (Shoji et al., 1999).

Swordfish larvae grow rapidly, faster than other larval fishes with reportedly rapid growth. Growth rates reported in the present study are for larvae that have survived predation and possibly variable feeding success; these rates do not necessarily represent average larval growth of the overall population. Growth rates of larvae >11 mm PSL (13 DAH), 5.6 mm/d, are nonetheless faster than the larval growth rates of other fast-growing larvae that survive in the sea, e.g. sablefish (*Anoplopoma fimbria*) (Boehlert and Yoklavich, 1985), and the oceanic-pelagic common dolphinfish (*Coryphaena hippurus*) reared in the laboratory at high food densities without predation (Hassler and Hogarth, 1977; Kraul, 1991). The growth rate of larval swordfish <13 mm LJFL, 3 mm/d, is slower than the maximum (16 mm LJFL/d) and sustained (10 mm LJFL/d) growth

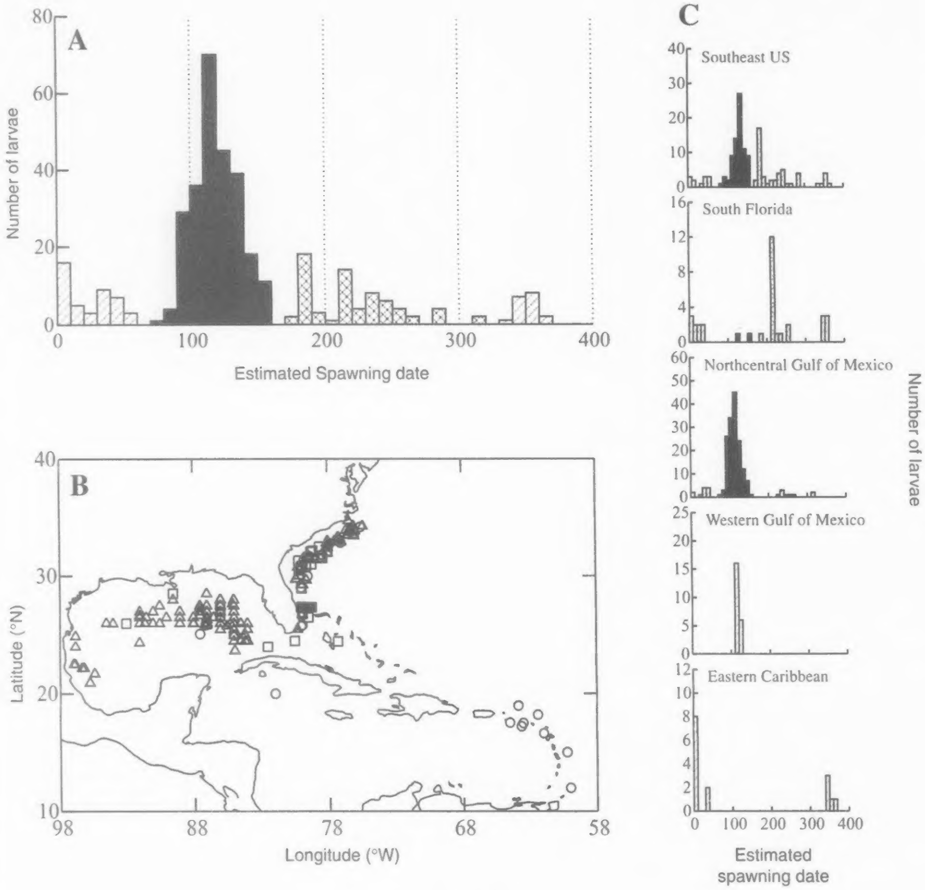


Figure 5

Estimated spawning dates (day of the year) for swordfish larvae collected from the Caribbean Sea, the Gulf of Mexico, and off the southeast coast of the United States (hatched bars and circles depict winter in the northern hemisphere, filled bars and triangles spring-summer, and cross-hatched bars and squares summer-fall): (**A**) number of larvae and estimated spawning dates; (**B**) geographical distribution of larval collections; (**C**) number of larvae and estimated spawning date by region; (**D**) number of larvae by region and season.

rate of larval blue marlin over the first 100 days or <1000 mm LJFL (Prince et al., 1991).

Larval and juvenile swordfish from the western North Atlantic and Mediterranean exhibit four growth phases. Growth is linear for larvae <11 mm PSL, for larvae 11 to 115 PSL, and for juveniles 510 to 740 mm LJFL (Megalofonou et al., 1995). Growth becomes allometric for larger juveniles (Ehrhardt, 1992). Larvae <11 mm grow ~0.1 mm LJFL/d. After the acceleration of growth, larvae in the western North Atlantic grow at ~3 mm LJFL/d, whereas young juveniles in the Mediterranean grow at 23 mm LJFL/d (Megalofonou et al., 1995). Growth slows in older juveniles <250 mm LJFL to ~2.5 mm LJFL/d (Ehrhardt, 1992). Adult growth (Berkeley and Houde, 1983; Tserpes and Tsimenides, 1989) may constitute a fifth phase, as recognized by Yabe et al. (1959) for Pacific swordfish.

The limited diet of larval swordfish is unusual; few larval fishes prey almost exclusively upon either copepods or larval fishes. Swordfish larvae 12.0 mm total length (TL) eat zooplankton, and larger larvae >12.0 mm TL eat other fish larvae (Gorbunova and Lipskaya, 1975), including conspecifics (Arata, 1954). Larval fishes as a whole are selective feeders; *Corycaeus* is selected by larval percoids in the Gulf of Mexico (Govoni et al., 1986b). Young larval istiophorids from the Florida Current eat primarily cyclopoid copepods of the genera *Corycaeus*, *Farranula*, and *Oithona* (Post et al., 1997), before they become piscivorous (Gorbunova and Lipskaya, 1975). Closely related genera of fishes exhibit

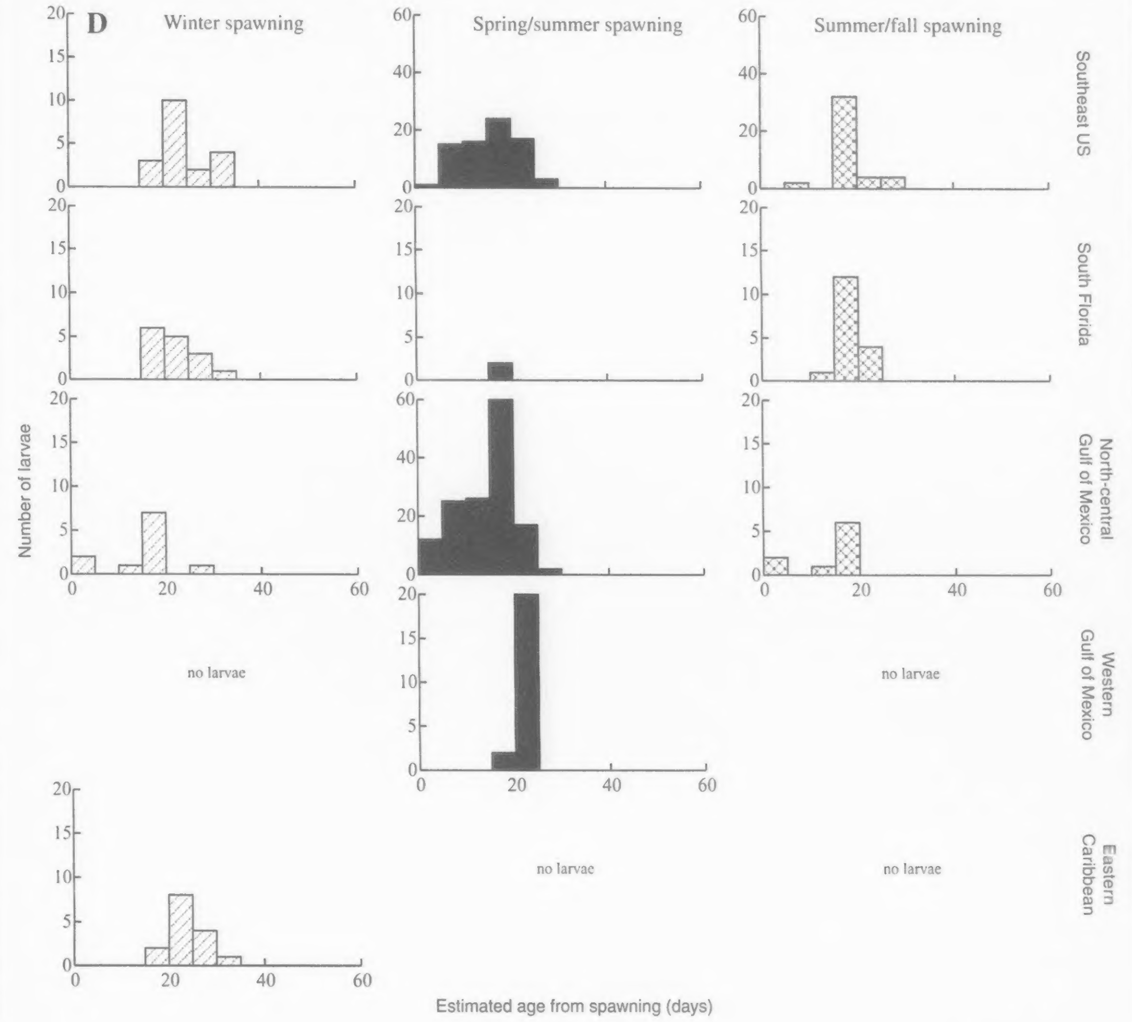


Figure 5 (continued)

different diets, even when occupying the same space (e.g. Govoni et al., 1983; 1986b). Larvae of the related istiophorids have limited diets, but these are not as exclusive or abruptly changing as that of swordfish. Diets of larvae examined in our study showed no evidence of cannibalism.

The diet of larval swordfish helps to resolve their vertical distribution. Most larvae have been collected at the surface in neuston or dip nets (Tåning, 1955; Yabe et al., 1959; Gorbunova, 1969; Nishikawa and Ueyanagi, 1975), although some have been collected in plankton nets that fished principally below the surface (Grall et al., 1983; Govoni et al., 2000). The diet of swordfish larvae indicates that larvae <11 mm PSL may live in the near surface water, whereas larvae >11 mm are neustonic. *Corycaeus* is a common neritic copepod of the Caribbean, the Florida Current, and the continental shelf off the southeast coast of the United States; *Corycaeus* is not neustonic (Owre and Foyo, 1967; 1972; Paffenhöfer, 1983; 1985). That *Corycaeus* is eaten almost exclusively by young swordfish larvae implies that these larvae occupy the near-surface pelagia. Istiophorid larvae undertake dietary shifts (Voss, 1953; Gorbunova and Lipskaya, 1975; Lipskaya and Gorbunova, 1977) and changes in vertical distribution (Bartlett and Haedrich, 1968; Leis et al., 1987) that are similar to those of swordfish larvae, but conflicting evidence exists for vertical distribution of larval istiophorids. Gorbunova and Lipskaya (1975) implied that istiophorid larvae accumulate in surface waters during the day and disperse below the surface at night, but Bartlett and Haedrich (1968) indicated the reverse. Large swordfish larvae are caught during the day and night in the neuston. The restricted diets of both large and small larvae implies little vertical movement.

The overall modal increase in larval age from the Gulf of Mexico to the north indicates that spawning takes place in the north-central Gulf of Mexico and off the southeast coast of the United States and that there is possible northward transport. Estimated ages, along with velocities and trajectories of currents, indicate that larvae could be transported from considerable distances, but only if they remain within the axes of major currents. The smallest larva collected off the Carolinas was 3.9 mm PSL and had an estimated age of 7 d AFH (this specimen was previously reported as being approximately 4 days old in Govoni et al. [2000]). With 3 days incubation at 25°C added to this estimated age, a swordfish egg and larva would be planktonic for 10 days. With a mean axial trajectory and velocity of the Florida Current and Gulf Stream of 1.5 m/s (Olson et al., 1994), a larva 3.9 mm PSL could be transported from as far away as 910 km, which could place the origin of this larva in the Straits of Florida, if its northward progress had not been checked by eddies so typical of the Gulf Loop Current (Maul and Vukovich, 1993) and Gulf Stream, particularly after the Gulf Stream exits the Straits of Florida (e.g. Lee et al., 1991; Govoni and Hare, 2001). Off the southeast coast of the United States, swordfish larvae aggregate in the western Gulf Stream frontal zone (Govoni et al., 2000), where northward current velocities are considerably slower than 1.5 m/s (Marmorino et al., 1999). Because larvae reside primarily in the western Gulf Stream frontal zone where northward velocities are slower and where the front itself is so frequently distorted by meanders and eddies, it is unlikely, but not impossible, that a larva as young as 7 d AFH collected off the Carolinas of the United States could have been transported from the Straits of Florida.

The largest and oldest larva examined, one collected off South Carolina (Govoni and Hare, 2001), was 115 mm PSL and had an estimated age of 30 d AFH; with 3 days incubation this fish could have been at large for 33 days and would have traveled 4290 km, given the mean axial trajectories and velocities of the Caribbean Sea (~0.2 m/s) and the Yucatan, Gulf Loop, and Florida Currents (~1.5 m/s). This calculation might place the spawning origin of this larva in the eastern Caribbean Sea or south of the Sargasso Sea if a direct, unchecked passage is assumed.

Inference of the seasonality and geography of spawning is limited and biased by the unsystematic temporal and spatial distribution of the present collections of larval swordfish and by uncertainties about the rate and trajectory of transport of eggs and larvae. Yet, taken as a whole, spawning dates, back-calculated from larvae collected in various regions of the western North Atlantic, and the abundance and spatial distribution of the youngest larvae indicate a spawning distribution with modes in three seasons and five regions. The western Gulf Stream frontal zone is the focus of spawning off the southeastern coast of the United States. Spawning in the Gulf of Mexico seems to be focused in the vicinity of the northern most arc of the Gulf Loop Current.

Estimated spawning dates and the spatial distribution of young larvae offer an alternative to gonad condition and oocyte status as a means of resolving spawning season and location. Spawning season and location resolved in the present study corroborate the scenario recently proposed by Arocha (1997). Rather than the single breeding unit currently recognized for the western North Atlantic by ICCAT, Chow and Takeyama (2000) and Arata (1997) proposed two spawning groups: one south of the Sargasso Sea and east of the Lesser Antilles, and the other in the Windward Passage of the Antilles, the Yucatan Channel, and the Straits of Florida up to 35°N latitude. Accordingly, spawning begins in December south of the Sargasso Sea. Larvae from this spawning group transit into the Caribbean, are retained there by its anticyclonic circulation, and use the southeastern Caribbean as a nursery area. Arocha (1997) implied that the second group spawns progressively later in the year and that larvae are transported with the Gulf Loop and Florida Currents, and the Gulf Stream. Arocha (1997) speculated that larvae and juveniles use the Gulf of Mexico and waters inshore of the Gulf Stream as a nursery area. Spawning dates and abundances of young larvae corroborate Arocha's (1997) proposed scenario for the seasonality and location of spawning and confirm spawning off the southeastern United States in the late spring and summer in the northern hermisphere. Spawning dates and abundance of young larvae also indicate the Gulf of Mexico as a nursery area. Further, large numbers of juveniles discarded from the longline fishery prosecuted in the vicinity of the Charleston Gyre (Cramer, 2001) and the collection of larvae there (Govoni and Hare, 2001) indicate that the waters off the southeastern coast of the United States serve as a nursery area.

Swordfish larvae are collected elsewhere in the western North Atlantic, although they are rarely caught north of Cape Hatteras, North Carolina (Tibbo and Lauzier, 1969). The trajectory of the Gulf Stream north of Cape Hatteras is convoluted and its velocity is slower, ~1 m/s (e.g. Bowers and Rossby, 1989; Flierl and Davis, 1993; Hare et al., 2002); the transit period of plankton from Cape Hatteras to the Azores is 120–300 days (Scheltema, 1971). Swordfish spawned in the western North Atlantic would be juvenile fish by the time they reached the eastern North Atlantic. North of Cape Hatteras, the Gulf Stream sheds eddies into the Sargasso Sea (McGuillicuddy et al., 1998); thus, the general location for juvenile swordfish that are spawned and not retained in the western North Atlantic may well be the central Atlantic and Sargasso Sea.

Swordfish are multiple spawners (Arocha, 2002) and adults may move and spawn among regions of the western North Atlantic. Movement of spawning adults, along with transport of larvae, may result in the genetically well-mixed population of the western North Atlantic (Alvarado Bremer et al., 1995a).There is apparently no genetic exchange between northwestern Atlantic and Mediterranean reproductive populations (Alvarado Bremmer et al., 1995b; Chow and Takeyama, 2000). Transoceanic migration of adult fish is possible, but cross-Atlantic transfer of swordfish larvae is not likely. Swordfish larvae, collected principally in water ≥25°C (Govoni et al., 2000), probably perish as Gulf Stream water cools when it traverses the northern western North

Atlantic (Scheltema, 1971; Cowen et al., 2000; Gaylord and Gaines, 2000). Larvae that are not retained by eddies of the Gulf Loop Current and the Gulf Stream may be diverted to the central Atlantic (McGuillicuddy et al., 1998) and probably do not transit to the eastern North Atlantic.

Acknowledgments

We thank L. R. Settle [NOAA - NOS, Center for Fisheries and Habitat Research (CCFHR)] for the collection of some of the swordfish specimens reported in our study. M. M. Leiby (Florida Department of Natural Resources) loaned SEAMAP collections. F. Arocha (Universidad de Oriente, Venezuela), S. A. Berkeley (Oregon State University), and D. L. Lee and J. L. Cramer (NOAA-Fisheries, Southeast Fisheries Science Center) provided invaluable counsel on swordfish biology. D. W. Ahrenholz and M. H. Prager (CCFHR, National Marine Fisheries Service, NOAA), W. J. Richards (Fisheries, Southeast Fisheries Science Center, NOAA), and F. Arocha provided reviews of the manuscript.

Literature cited

Alvarado Bremer, J. R., A. J. Baker, and J. Mejuto.
1995a. Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (*Xiphias gladius*) in the Atlantic Ocean. Can. J. Fish. Aquat. Sci. 52:1720–1732.

Alvarado Bremer, J. R., J. Mejuto, T. W. Greig, and B. Ely.
1995b. Global population structure of the swordfish (*Xiphias gladius*) as revealed by analysis of the mitochondrial DNA control region. J. Exp. Mar. Biol. Ecol. 197:295–310.

Arata, G. F.
1954. A contribution to the life history of the swordfish, *Xiphias gladius* Linnaeus, from the south Atlantic coast of the United States and the Gulf of Mexico. Bull. Mar. Sci. Gulf Caribb. 4:183–243.

Arocha, F.
1997. The reproductive dynamics of swordfish *Xiphias gladius* L. and management implications in the northwestern Atlantic. Ph.D. diss., 350 p. Univ. Miami, Miami, FL.
2002. Oocyte development and maturity classification of swordfish from the north-western Atlantic. J. Fish Biol. 60:13–27.

Bartlett, M. R., and R. L. Haedrich.
1968. Neuston nets and South Atlantic larval blue marlin (*Makaira nigricans*). Copeia 1968:469–474.

Beckett, J. S.
1975. Biology of swordfish, *Xiphias gladius* L., in the northwest Atlantic ocean. *In* Proceedings of the international billfish symposium Kailua-Kona, Hawaii, 9–12 August 1972, part 1: Report of the symposium (R. S. Shomura and F. Williams, eds.), p. 103–106. NOAA Tech. Rep. NMFS-SSRF-675.

Berkeley, S. A.
1983. Atlantic swordfish stock structure data and suggestions for its interpretation. Int. Comm. Conserv. Atl. Tuna, Collect. Vol. Sci. Pap. 18:839–845.

Berkeley, S. A., and E. D. Houde.
1983. Age determination of broadbill swordfish, *Xiphias gladius*, from the Straits of Florida, using anal fin spine sections. *In* Proceedings of the international workshop on age determination of oceanic pelagic fishes: tunas, billfishes, and sharks (E. D. Prince and L. M. Pulos, eds.), p. 137–143. NOAA Tech. Rep. NMFS-SSRF-8.

Boehlert, G. W., and M. M. Yoklavich.
1985. Larval and juvenile growth of sablefish, *Anoplopoma fimbria*, as determined from otolith increments. Fish. Bull. 83:475–481.

Boicourt, W. C., W. J. Wiseman Jr., A. Valle-Levinson, and L. P. Atkinson.
1998. Continental shelf of the southeastern United States and the Gulf of Mexico: in the shadow of the western boundary current. *In* The sea (A. R. Robinson and K. H. Brink, eds.), vol. 11, p. 135–183. John Wiley & Sons, New York, NY.

Bowers, A. S., and T. Rossby.
1989. Evidence of cross-frontal exchange processes in the Gulf Stream based on isopycnal RAFOS float data. J. Phys. Oceanogr. 19:1177–1190.

Campana, S. E.
2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish Biol. 59:197–242.

Chow, S., and H. Takeyama.
2000. Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish. J. Fish Biol. 56:1087–1098.

Collette, B. B., T. Potthoff, W. J. Richards, S. Ueyanagi, J. L. Russo, and Y. Nishikawa.
1984. Scombroidei: development and relationships. *In* Ontogeny and systematics of fishes (H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, and S. L. Richardson, eds.), p. 591–620. Am. Soc. Ichthyol. Herpetol. Spec. Publ. 1.

Cowen, R. K., K. M. M. Lwiza, S. Sponaugle, C. B. Paris, and D. B. Olson.
2000. Connectivity of marine populations: open or closed? Science 287:857–859.

Cramer, J.
2001. Geographic distribution of longline effort and swordfish discard rates in the Straits of Florida and oceanic waters of the continental shelf, slope, and Blake Plateau off Georgia and the Carolinas from 1991 to 1995. *In* Island in the stream: the ecology and fisheries of the Charleston Bump (G. R. Sedberry, ed.), p. 97–103. Am. Fish. Soc. Symp. 25.

Ehrhardt, N. M.
1992. Age and growth of swordfish, *Xiphias gladius*, in the northwestern Atlantic. Bull. Mar. Sci. 50:292–301.

Flierl, G. R., and C. S. Davis.
1993. Biological effects of Gulf Stream meandering. J. Mar. Res. 51:529–560.

Forbes, T. L., and G. R. Lopez.
1989. Determination of critical periods in ontogenetic trajectories. Funct. Ecol. 3:525–632.

Gaylord, B., and S. D. Gaines.
2000. Temperature or transport? Range limits in marine species mediated solely by flow. Am. Nat. 155:769–789.

Gorbunova, N. N.
1969. Breeding grounds and food of the larvae of the swordfish *Xiphias gladius* Linneus (Pisces, Xiphiidae). Probl. Ichthyol. 9:375–387.

Gorbunova, N. N., and N. Ya. Lipskaya.
1975. Feeding of larvae of the blue marlin, *Makaira nigricans* (Pisces, Istiophoridae). J. Ichthyol. 15:95–101.

Govoni, J. J., G. W. Boehlert, and Y. Watanabe.
1986a. The physiology of digestion in fish larvae. Environ. Biol. Fishes 16:59–77.

Govoni, J. J., and J. A. Hare.
2001. The Charleston Gyre as spawning and larval nursery habitat for fishes. *In* Island in the stream: the ecology and fisheries of the Charleston Bump (G. R. Sedberry, ed.), p. 123–136. Am. Fish. Soc. Symp. 25.

Govoni, J. J., D. E. Hoss, and A. J. Chester.
1983. Comparative feeding of three species of larval fishes in the northern Gulf of Mexico: *Brevoortia patronus*, *Leiostomus xanthurus*, and *Micropogonias undulatus*. Mar. Ecol. Prog. Ser. 13:189–199.

Govoni, J. J., P. B. Ortner, F. Al-Yamani, and L. Hill.
1986b. Selective feeding of spot, *Leiostomus xanturus*, and Atlantic croaker, *Micropogonias undulatus*, larvae in the northern Gulf of Mexico. Mar. Ecol. Prog. Ser. 28: 175–183.

Govoni, J. J., B. W. Stender, and O. Pashuk.
2000. Distribution of larval swordfish, *Xiphias gladius*, and probable spawning off the southeastern United States. Fish. Bull. 98:64–74.

Grall, C., D. P. de Sylva, and E. D. Houde.
1983. Distribution, relative abundance, and seasonality of swordfish larvae. Trans. Am. Fish. Soc. 112:235–246.

Hare, J. A., J. H. Churchill, R. K. Cowen, T. J. Berger, P. C. Cornillon, P. Dragos, S. M. Glenn, J. J. Govoni, and T. N. Lee.
2002. Routes and rates of larval fish transport from the southeast to the northeast United States continental shelf. Limnol. Oceangr. 47: 1774–1789.

Hare, J. A., and R. K. Cowen.
1995. Effects of age, growth rate, and ontogeny on the otolith size–fish size relationship in bluefish, *Pomatomus saltatrix*, and the implications for back-calculation of size in fish early life history stages. Can. J. Fish. Aquat. Sci. 52:1909–1922.

Hassler, W. W., and W. T. Hogarth.
1977. The growth and culture of dolphin, *Coryphaena hippurus*, in North Carolina. Aquaculture 12:115–122.

Hitchcock, G. L., T. Rossby, J. L. Lillibridge, E. J. Lessard, E. R. Levine, D. N. Connors, K. Y. Borsheim, and M. Mork.
1994. Signatures of stirring and mixing near the Gulf Stream front. J. Mar. Res. 52:797–836.

Jones, C.
1986. Determining age of larval fish with the otolith increment technique. Fish. Bull. 84:91–104.

Kaji, T., M. Tanaka, M. Oka, H. Takeuchi, S. Ohsumi, K. Teruya, and J. Hirokawa.
1999. Growth and morphological development of laboratory-reared yellowfin tuna *Thunnus albacares* larvae and early juveniles, with special emphasis on the digestive system. Fish. Sci. 65:700–707.

Kendall, A. W., Jr., E. H. Ahlstrom, and H. G. Moser.
1984. Early life stages of fishes and their characters. *In* Ontogeny and systematics of fishes (H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, and S. L. Richardson, eds.), p. 11–22. Am. Soc. Ichthyol. Herpetol., Spec. Publ. 1.

Kotoulas, G., A. Magoulas, N. Tsimenides, and E. Zouros.
1995. Marked mitochondril DNA differences between Mediterranean and Atlantic populations of the swordfish, *Xiphias gladius*. Mol. Ecol. 4:473–481.

Kraul, S.
1991. Larviculture of the mahimahi, *Coryphaena hippurus*, in Hawaii, USA. J. World Aquacult. Soc. 24:410–421.

LaMonte, F.
1944. Note on breeding grounds of blue marlin and swordfish off Cuba. Copeia 4:258.

Lee, T. N., J. A. Yoder, and L. P. Atkinson.
1991. Gulf Stream frontal eddy influence on productivity of the southeast U.S. continental shelf. J. Geophys. Res. 96: 22,191–22,205.

Leis, J. M., B. Goldman, and S. Ueyanagi.
1987. Distribution and abundance of billfish larvae (Pisces: Istiophoridae) in the Great Barrier Reef lagoon and Coral Sea near Lizard Island, Australia. Fish Bull. 85:757–766.

Lipskaya, N. Ya., and N. N. Gorbunova.
1977. Feeding of sailfish larvae. Oceanology 17:340–344.

Markle, G. E.
1975. Distribution of larval swordfish in the Northwest Atlantic Ocean. *In* Proceedings of the international billfish symposium Kailua-Kona, Hawaii, 9–12 August 1972, part 1: Report of the symposium (R. S. Shomura and F. Williams, eds.), p. 252–260. NOAA Tech. Rept. NMFS-SSRF-675.

Marmorino, G. O., D. R. Lyzenga, and J. A. C. Kaiser.
1999. Comparison of airborne synthetic aperture radar imagery with in situ surface-slope measurements across Gulf Stream slicks and a convergent front. J. Geophys. Res. 104:1405–1422.

Maul, G. A., and F. M. Vukovich.
1993. The relationship between variations in the Gulf of Mexico Loop Current and Straits of Florida volume transport. J. Phys. Oceanogr. 23:785–796.

McGowan, C.
1988. Differential development of the rostrum and mandible of the swordfish (*Xiphias gladius*) during ontogeny and its possible functional significance. Can. J. Zool. 66:496–503.

McGuillicuddy, D. J., A. R. Robinson, D. A. Siegel, H. J. Jannasch, R. Johnson, T. D. Dickey, J. McNiel, A. F. Michaels, and A. H. Knap.
1998. Influence of mesoscale eddies on new production in the Sargasso Sea. Nature 395:263–266.

Megalofonou, P., J. M. Dean, G. DeMetrio, C. Wilson, and S. Berkeley.
1995. Age and growth of juvenile swordfish, *Xiphias gladius* Linnaeus, from the Meditteranean Sea. J. Exp. Mar. Biol. Ecol. 188:79–88.

Mooers, C. N. K., and G. A. Maul.
1998. Intra-Americas sea circulation: coastal segment (3,W). *In* The sea (A. R. Robinson and K. H. Brink, eds.), vol. 11, p. 183–208. John Wiley & Sons, New York, NY.

Nishikawa, Y., and S. Ueyanagi.
1975. The distribution of the larvae of swordfish, *Xiphias gladius*, in the Indian and Pacific oceans. *In* Proceedings of the international billfish symposium Kailua-Kona, Hawaii, 9–12 August 1972, part 1: Report of the symposium (R. S. Shomura and F. Williams, eds.), p. 261–264. NOAA Tech. Rep. NMFS-SSRF-675.

Olson, D. B., G. L. Hitchcock, A. J. Mariano, C. J. Ashjian, G. Peng, R. W. Nero, and G. P. Podestá.
1994. Life on the edge: marine life and fronts. Oceanography 7:52–60.

Owre, H. B., and M. Foyo.
1967. Copepods of the Florida Current. Fauna Caribaea 1, Crustacea, part 1: Copepoda, 137 p. Inst. Mar. Sci., Univ. of Miami, Miami, FL.
1972. Studies on Caribbean zooplankton. Description of the program and results of the first cruise. Bull. Mar. Sci. 22: 483–521.

Paffenhöfer, G.-A.
1983. Vertical zooplankton distribution on the northeastern Florida shelf and its relation to temperature and food abundance. J. Plankton Res. 5:15–33.

1985. The abundance and distribution of zooplankton on the southeastern shelf of the United States. *In* Oceanography of the southeastern U.S. continental shelf (L. P. Atkinson, D. W. Menzel, and K. A. Bush, eds.), p. 104–117. Am. Geophysical Union, Washington, DC.

Palko, B. J., G. L. Beardsley, and W. J. Richards.
1981. Synopsis of the biology of the swordfish, *Xiphias gladius* Linnaeus, 21 p. NOAA Tech. Rep. NMFS Circ. 441.

Post, J. T., J. E. Serafy, J. S. Ault, T. R. Capo, and D. P. de Sylva.
1997. Field and laboratory observations on larval Atlantic sailfish (*Istiophorus platypterus*) and swordfish (*Xiphias gladius*). Bull. Mar. Sci. 60:1026–1034.

Potthoff, T., and S. Kelley.
1982. Development and structure of the vertebral column, fins and fin supports, branchiostegal rays and squamation in the swordfish *Xiphias gladius*. Fish. Bull. 80:161–186.

Prince, E. D., D. W. Lee, J. R. Zweifel, and E. B. Brothers.
1991. Estimating age and growth of young Atlantic blue marlin *Makaira nigricans* from otolith microstructure. Fish. Bull. 89:441–459.

Reeb, C. A., L. Arcangeli, and B. A. Block.
2000. Structure and migration corridors in Pacific populations of the swordfish *Xiphius gladius*, as inferred through analyses of mitochondrial DNA. Mar. Biol. 136:1123–1131.

Richards, W. J., and T. Potthoff.
1980. Larval distributions of scombrids (other than bluefin tuna) and swordfish in the Gulf of Mexico in the spring of 1977 and 1978. Int. Comm. Conserv. Atl. Tuna, Coll. Vol. Sci. Pap. 9:680–694.

Rogers, J. S., J. A. Hare, and D. G. Lindquist.
2001. Otolith record of age, growth, and ontogeny in larval and pelagic juvenile *Stephanolepis hispidus* (Pisces: Monacanthidae). Mar. Biol. 138:945–953.

Sanzo, L.
1910. Uovo e larva di Pesce-spada (*Xiphias gladius* L.). Riv. Mens. Pesca Idrobiol. 12:206–209.

Scheltema, R. S.
1971. The dispersal of the larvae of shoal-water benthic invertebrate species over long distances by ocean currents. *In* Fourth European marine biology symposium (D. J. Crisp, ed.), p. 7–28. Cambridge Univ. Press, Cambridge, UK.

Schmitz, W. J., J. R. Luyten, and R. W. Schmitt.
1993. On the Florida Current T/S envelope. Bull. Mar. Sci. 53:1048–1065.

Secor, D. H., J. M. Dean, and E. H. Laban.
1991. Manual for otolith removal and preparation for microstructural examination, 85 p. Electric Power Research Institute and the Belle W. Baruch Institute for Marine Biology and Coastal Research, Univ. South Carolina, Columbia, SC.

Shoji, J., T. Maehara, and M. Tanaka.
1999. Short-term occurrence and rapid growth of Spanish mackerel larvae in the central waters of the Seto Inland Sea, Japan. Fish. Sci. 65:68–72.

Tåning, Å.
1955. On the breeding areas of the swordfish (*Xiphias*). Deep-Sea Res. 3(suppl.):438–450.

Taylor, R. G., and M. D. Murphy.
1992. Reproductive biology of the swordfish *Xiphias gladius* in the Straits of Florida and adjacent waters. Fish. Bull. 90:809–816.

Theilacker, G. H.
1980. Changes in body measurements of larval northern anchovy, *Engraulis mordax*, and other fishes due to handling and preservation. Fish. Bull 78:685–692.

Tibbo, S. N., and L. M. Lauzier.
1969. Larval swordfish (*Xiphias gladius*) from three localities in the western Atlantic. J. Fish. Res. Board Can. 26:3248–3251.

Tserpes, G., and N. Tsimenides.
1989. Age determination and growth of swordfish *Xiphias gladius* L., 1958 in the Aegean Sea. Fish Res. 8:159–168.

Voss, G. L.
1953. A contribution to the life history and biology of the sailfish, *Istiophorus americanus* Cuv. and Val., in Florida waters. Bull. Mar. Sci. Gulf Caribb. 3:206–240.

Wiebe, P. H., K. H. Burt, S. H. Boyd, and A. W. Morton.
1976. A multiple opening/closing net and environmental sensing system for sampling zooplankton. J. Mar. Sci. 34:313–326.

Yabe, H., S. Ueyanagi, S. Kikawa, and H. Watanabe.
1959. Study on the life-history of the sword-fish, *Xiphias gladius* Linnaeus. Rep. Nankai Reg. Fish. Res. Lab. 10:107–150.

Yasuda, F., H. Kohno, A. Yatsu, H. Ida, P. Arena, F. L. Greci, and Y. Taki.
1978. Embryonic and early larval stages of the swordfish, *Xiphias gladius*, from the Mediterranean. J. Tokyo Univ. Fish. 65:91–97.

Abstract—Although subsampling is a common method for describing the composition of large and diverse trawl catches, the accuracy of these techniques is often unknown. We determined the sampling errors generated from estimating the percentage of the total number of species recorded in catches, as well as the abundance of each species, at each increase in the proportion of the sorted catch. We completely partitioned twenty prawn trawl catches from tropical northern Australia into subsamples of about 10 kg each. All subsamples were then sorted, and species numbers recorded. Catch weights ranged from 71 to 445 kg, and the number of fish species in trawls ranged from 60 to 138, and invertebrate species from 18 to 63. Almost 70% of the species recorded in catches were "rare" in subsamples (less than one individual per 10 kg subsample or less than one in every 389 individuals).

A matrix was used to show the increase in the total number of species that were recorded in each catch as the percentage of the sorted catch increased. Simulation modelling showed that sorting small subsamples (about 10% of catch weights) identified about 50% of the total number of species caught in a trawl. Larger subsamples (50% of catch weight on average) identified about 80% of the total species caught in a trawl.

The accuracy of estimating the abundance of each species also increased with increasing subsample size. For the "rare" species, sampling error was around 80% after sorting 10% of catch weight and was just less than 50% after 40% of catch weight had been sorted. For the "abundant" species (five or more individuals per 10 kg subsample or five or more in every 389 individuals), sampling error was around 25% after sorting 10% of catch weight, but was reduced to around 10% after 40% of catch weight had been sorted.

Manuscript approved for publication 9 March 2003 by Scientific Editor.

Manuscript received 26 June 2003 at NMFS Scientific Publications Office.

Fish Bull. 101:790–799 (2003).

Does the size of subsamples taken from multispecies trawl catches affect estimates of catch composition and abundance?

Donald S. Heales
David T. Brewer
CSIRO Marine Research
233 Middle St.
Cleveland, Queensland 4163, Australia
E-mail address (for D. S. Heales): don.heales@marine.csiro.au

You-Gan Wang
Dept. of Biostatistics
Harvard University
Boston, Massachusetts 02115

Peter N. Jones
CSIRO Mathematical and Information Sciences
233 Middle St.,
Cleveland, Queensland 4163, Australia

Concerns are held worldwide regarding the sustainability of bycatch species taken in trawls, particularly prawn trawls. Under the voluntary FAO Code for Responsible Fisheries, managers are required to "take measures to conserve target species, associated or dependent species and nontarget species and their environment" (FAO, 1995). An essential part of this process is the accurate monitoring of population sizes and structures.

With large trawl catches, subsampling is often the only cost-effective or feasible way to describe the bycatch composition. How well these subsamples represent the total catch depends on how diverse the catch is, how well the catch is mixed before the subsamples are taken, and what proportion of the catch is taken as a subsample.

There is a large literature on subsampling theory for terrestial insect studies (Van Ark and Meiswinkel, 1992), aquatic macroinvertebrate studies (Vinson, 1996; Walsh, 1997), and marine ecological studies (Andrew and Mapstone, 1987). However, in most of these studies, samples of very small animals collected in the field can be resuspended in fluid and mixed evenly in the laboratory before the subsamples are taken. In fisheries, in direct contrast, large catches are extremely difficult to manipulate and redistribute evenly before subsampling. A few fisheries studies have examined the impact of subsampling on estimates of the abundance and different size ranges of one or a few dominant species. For example, in the *Crangon* trawl fisheries in Belgian waters, sampling strategy had only a minor effect on the reliability of estimates of size selectivity for the targeted shrimp (Polet and Redant, 1999). In UK waters, subsampling trawled fish (both target species and discards) from either the sorting conveyor or the pound made no difference to catch composition estimates (Tamsett et al., 1999).

However, in tropical trawl fisheries, over one hundred species can be recorded in a single catch. Under ESD (ecological sustainable development) guidelines, all these species (both target and bycatch) are equally important but there has been very little research on subsampling techniques applicable to such diverse catches. A recent study in Australia's Northern Prawn Fishery (NPF) examined the accuracy of subsampling from large, diverse catches of fish and invertebrates (Heales et al., 2000). For most of the "abundant" species, their position on trawler sorting trays from which the bycatch subsamples were collected, had little effect on the accuracy in representing the catches.

Although the accuracy of subsampling is a general problem for all multispecies fisheries, few other studies have been published on the topic. Reliable techniques for subsampling are needed, however, especially with the demands that bycatch species, as well as the target species, should be ecologically sustainable. We describe here research done in Australia's NPF but the results and methods are applicable to many sampling problems in fisheries.

The NPF is a large tropical trawl fishery that extends from Cape York in Queensland to Cape Londonderry in Western Australia. In addition to catching penaeid prawns (e.g. 8531 metric tons (t) in 1997–98, Taylor and Die, 1999), its bycatch component is estimated at over 38,000 t a year (Pender et al., 1992) or more than 80% of the total catch in the tiger prawn fishery (Brewer et al., 1998). The neighboring Torres Straits Prawn Fishery (TSPF) also has an estimated annual bycatch of 4800 t (Williams, 1985).

The NPF has a management requirement to assess the impact of trawling on nontarget species. The bycatch of both these prawn trawl fisheries (NPF and TSPF) is very diverse. Ramm et al. (1990) recorded 115 fish taxa in their study of NPF waters, and Brewer et al. (1998) recorded over 250 species from one area of the NPF. At least 390 fish species, 234 invertebrate taxa, and 43 elasmobranch species have been recorded in a current bycatch project in the NPF (Stobutzki et al., 2000).

Despite the lack of knowledge on the ability of subsamples to accurately represent such diverse catches, many studies of trawl communities in Northern Australia have used subsampling techniques to estimate catch rates. In two bycatch studies of the NPF, the smaller catches were entirely sorted and the larger catches subsampled (Poiner and Harris, 1986; Harris and Poiner, 1991). Trawl catches in another bycatch study were spread evenly over the sorting tray and a visually estimated fraction of the catch was subsampled (Ramm et al., 1990). Other workers simply subsampled without confirming the accuracy of their subsampling techniques (Blaber et al., 1990 and 1994; Martin et al., 1995; Brewer et al., 1998; Wassenberg et al., 1998).

A lack of knowledge of trawl impacts on nontarget species has led to the present CSIRO study that describes the bycatch from the NPF and provides a framework for any future bycatch monitoring program. As part of that study, we made the first assessment of the accuracy of subsampling over a range of subsample sizes as a tool for estimating the total catch composition of a large multispecies fishery.

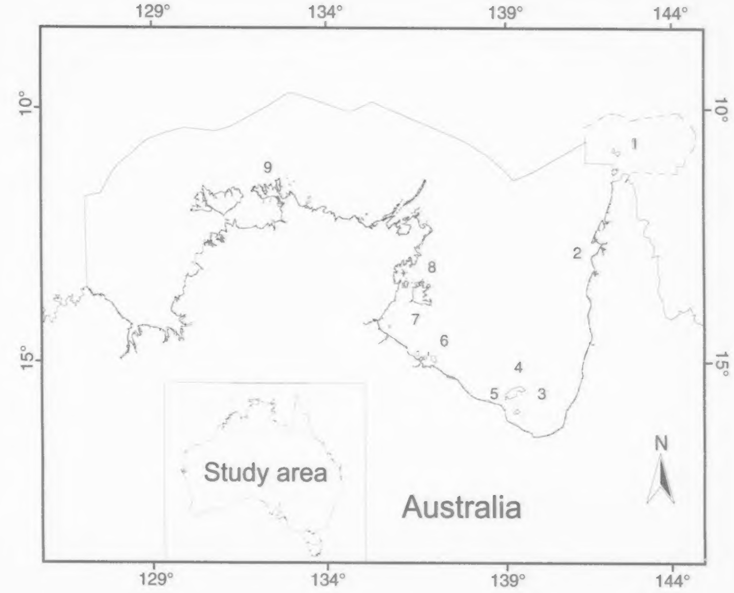


Figure 1

Map of the Torres Straits Fishery (area 1—broken line) and Northern Prawn Fishery (unbroken line), Australia, showing the managed area of the fisheries and the regions where samples were collected. Regional codes are 1 = Torres Straits, 2 = Weipa, 3 = east of Mornington Island, 4 = north of Mornington Island, 5 = west of Mornington Island, 6 = east of Vanderlin Islands, 7 = south of Groote Eylandt, 8 = north of Groote Eylandt, 9 = Melville Island.

Materials and methods

Data were collected from a series of 14 trawl samples taken during two research cruises of the RV *Southern Surveyor*. These samples were collected from one region of the Torres Straits Prawn Fishery (TSPF), and from eight of the major tiger prawn (*Penaeus esculentus* and *P. semisulcatus*) fishing regions of the Northern Prawn Fishery (NPF), (namely Weipa, east of Mornington Island, north of Mornington Island, west of Mornington Island, north of Vanderlin Islands, south of Groote Eylandt, north of Groote Eylandt, and Melville Island) (Fig. 1). All trawls were undertaken in either late summer 1997 (February–March, the end of the wet season) or in mid-spring 1997 (September–October, the dry season). We used a single 14-fathom Florida-Flyer prawn trawl net so that the data would be comparable with data from either of the two nets used by the twin-rigged commercial NPF vessels in the tiger prawn fishery. All trawls were done at night, again to be comparable with the fishery. Duration of trawls ranged from 1 to 3 hours (Table 1), and depths ranged from 23 to 42.3 m.

A further six trawl catches were sampled by a scientific observer on board commercial NPF vessels fishing for tiger prawns north of Mornington Island in late May 1997, and north of Groote Eylandt in late September 1997. Each trawl sample consisted of the entire catch from one of the two 14-fathom Florida-Flyer prawn trawl nets used by these vessels. All trawls were done at night. Their duration

Table 1

Summary of catch data for 20 entirely sorted trawls from the Northern Prawn Fishery and Torres Straits Prawn Fishery. "Bycatch individuals" refers to the total number of bycatch animals (fish and invertebrates) in each trawl. "Fish species" refers to the total number of fish species recorded in the bycatch of each trawl. (*n*) is the total number.

Region	Duration of trawl (h)	Start time of trawls	Catch weight (kg)	Subsamples (*n*)	Bycatch individuals (*n*)	Fish species (*n*)	Invertebrate species (*n*)	All species (*n*)
Torres Straits	1.2	0400	94	9	4151	74	21	95
Weipa	2.7	0220	166	16	4911	92	36	128
East of Mornington Island	1.0	0415	274	24	7313	90	18	108
East of Mornington Island	2.9	0250	87	8	2019	77	45	122
North of Mornington Island	3.3	1845	182	16	9762	101	36	137
North of Mornington Island	3.0	2215	194	17	11015	94	42	136
North of Mornington Island	3.2	1840	445	36	13,826	114	52	166
North of Mornington Island	2.0	0350	71	7	1771	60	30	90
North of Mornington Island	2.2	0330	100	10	5067	77	34	111
West of Mornington Island	1.6	0415	174	16	5976	100	21	121
West of Mornington Island	1.7	0400	269	26	6967	87	24	111
North of Vanderlin Islands	2.7	0310	156	15	7182	71	19	90
South of Groote Eylandt	2.0	0345	315	27	23,751	105	25	130
South of Groote Eylandt	2.7	0250	165	17	3856	64	25	89
North of Groote Eylandt	2.5	2230	147	12	5558	68	28	96
North of Groote Eylandt	3.0	0215	85	9	2792	60	34	94
North of Groote Eylandt	3.5	1830	158	13	5664	89	43	132
North of Groote Eylandt	3.5	2215	169	15	8289	96	63	159
North of Groote Eylandt	3.5	2215	189	16	5748	96	43	139
Melville Island	3.0	0130	170	14	4635	60	30	90
Total			3610	323	140,253			

ranged from 3 to 3.5 hours (Table 1), and depths ranged from 29 to 41 m.

Sample collection

On the research vessel, catches were spilled from the codend onto the flat deck (equivalent to the sorting tray on commercial vessels). The entire catch of each trawl was progressively partitioned by shovelling the catch into consecutively numbered boxes (subsample replicates), each of about 10 kg (according to the methods described by Heales et al., 2000). Partitioning of the catch began at the outer edges and continued in a clockwise direction, and subsamples were taken from each of four major compass directions: north, east, south, and west until the entire catch had been collected in successively numbered boxes. The direction of the ship's bow was always designated as north.

Samples on the research vessel were sorted immediately. Fish and invertebrates were identified to the lowest taxonomic level possible (mostly species). Where this was not possible, the data were grouped to genus and in a few cases to family. Total numbers and weights were recorded for each species in each subsample and entered directly into a relational database.

On the commercial vessels, the catches were spilled onto the sorting tray and the commercial-size prawns were removed. The bycatch would then normally move down a trash chute and spill overboard. However, to sample a catch, the trash chute was diverted so that all the bycatch was collected in consecutively numbered boxes (subsample replicates) each of approximately 10 kg.

All samples collected from commercial vessels were frozen on board and transported to the laboratory for subsequent sorting, identification, and data entry according to the methods described above.

Although most bycatch species were identified to species level, some could only be identified to genus and in a few cases to family. In order to be consistent in terms used throughout this study, we use the term species (plural form) even when referring to multispecies groups.

The methods used to collect subsamples on both the research and commercial vessels differed only in the position from which subsamples were taken (see earlier "Material and methods" section). However, a previously published study (Heales et al., 2000) showed that the majority of the "abundant" bycatch species were evenly distributed throughout the catch. Consequently, for all analyses we combined the 14 catches collected from the research vessel with the six catches from commercial vessels.

Data analysis

Abundance groupings Within each catch, there was a large range of species (both fish and invertebrates) and they occurred at many different levels of relative abundance. To obtain an overview of how rarely, or how frequently, different species occurred in catches, we reduced each occurrence of a species in a catch to an index of relative abundance. We concentrated solely on determining the accuracy of taking different size subsamples in representing the range of relative abundances (from very low to very high) of the species in these catches.

The relative abundance indices were based on the average number of individuals of a given species that were recorded in a standardized 10-kg subsample taken from that catch. To generate this index, we used the following equation:

$$n = 10 \times (TotNum \,/\, Weight), \qquad (1)$$

where n = the mean number of individuals of a given species per 10-kg subsample;
$TotNum$ = the total number of individuals of that species in the whole catch; and
$Weight$ = the total weight of the catch in kg.

We derived a separate index of abundance for each species in every catch where it was recorded. Thus, a species that occurred in all 20 catches would have 20 different abundance indices in the analysis.

To highlight the differences in distribution between the two extremes of the "rare" species and the "abundant" species when estimating catch composition, we grouped the indices of abundance into 11 categories, ranging from less than one individual per 10-kg subsample, up to 10 or more individuals per subsample. Species with abundance indices of less than one individual per 10-kg subsample were classed as "rare"; those with one to less than five individuals were classed as "common"; and those with five or more individuals per 10-kg subsample were classed as "abundant."

For example, the common ponyfish (*Leiognathus moretoniensi*), was classed as "abundant" in 11 of the 20 catches, as "common" in eight catches, and "rare" in one catch. Because a species could have different abundance indices in each catch, individual species are not referred to by name in the results. Instead, we refer to the occurrence of each species in a catch, as one *case* of some relative abundance index that was recorded in that catch (i.e. one "case" of species by trawl abundance). The relative frequency of all the cases (i.e. abundance indices) in each of the three abundance categories (throughout the combined 20 catches) was then calculated.

To calculate the average number of bycatch individuals per 10-kg subsample (over all the catches), the following equation was used:

$$X = 10 \times (Total\ number \,/\, Total\ weight) \qquad (2)$$

where X = the mean number of bycatch individuals (per 10-kg subsample);
Total number = the total number of all bycatch individuals (summed over all 20 catches); and
Total weight = the total weight (kg) of all bycatch individuals (summed over all 20 catches).

We then examined the average occurrence ratios within 10-kg subsamples for the "rare," "common," and "abundant" species.

Catch composition To examine the relationship between the number of recorded species and the weight of sorted catch, the subsamples were first analyzed in the order that they were collected. The cumulative number of species (both fish and invertebrates) was plotted against the cumulative weight of sorted catch, for each of the 20 catches. Each catch was also summarized in terms of the percentage of species recorded for each 10% increment of weight of sorted catch.

The order (position on the sorting tray) where the subsamples were collected on both the research and commercial vessels was just one of the many possible ways that a catch can be divided into 10-kg subsamples. To determine the level of accuracy in recording the number of species in a catch, we examined 200 combinations of subsample selection (with no replacement), by randomly reordering the subsamples using Monte Carlo simulations for each catch. We also calculated the cumulative number and percentage of species recorded, as well as the cumulative weight and percentage of the sorted catch, for each catch. The proportion of species recorded was fitted as a power function of the proportion of the weight of sorted catch, as described by the following asymptotic equation (Snedecor and Cochran, 1980):

$$y = p^k + \varepsilon, \qquad (3)$$

where y = the proportion of species recorded;
p = the proportion of the weight of catch sorted;
k = the mean exponential parameter; and
ε = the random normal error term, with unequal variance.

The variance of ε is assumed to be $p\,(1-p)\,\sigma^2$ to ensure that the variance of y is fixed at zero when $p = 0$ and 1. This formulation has the property that, when none of the catch has been sorted, no species will have been recorded. It also ensures that $y = 1$ when $p = 1$, i.e. when all the catch has been sorted, all of the species have been recorded. The estimate of σ^2 was obtained by fitting the following model according to the SAS procedure NLIN (version 7, SAS Inst., Cary NC):

$$y^* = p^k \,/\, \sqrt{(p(1-p))} + \varepsilon^*, \qquad (4)$$

where $y^* = y \,/\sqrt{(p(1-p))}$ and $\varepsilon^* = \varepsilon / \sqrt{(p(1-p))}$; and
ε^* now has homogeneous variance structure.

Different k values were estimated for each catch to reflect the variation in the relationship. The mean k_i value for a given catch (i=1–20) was obtained from 200 analyses for that catch. The predicted y values i.e. $\bar{y}_p$ (at p=0.1, 0.2 etc. to 1.0) were obtained by averaging p^{k_i} values across the 20 catches (note that this is different from $p^{\bar{k}}$ where $\bar{k}$ is the mean k value for the 20 catches). We defined the $\bar{y}_p$ values as the predicted expected proportion of species recorded after p proportion of catches had been sorted.

The corresponding 95% confidence interval for the predicted mean values ($\bar{y}_p$) was evaluated by using the width 1.96 σ_m where σ_m^2 is the variance of $\bar{y}_p$ given by

$$\begin{aligned}\sigma_m^2 &= V(p^k) + V(\varepsilon)\\ &= \left(\log(p)\bar{y}_p\sigma_k\right)^2 + p(1-p)\sigma^2,\end{aligned} \tag{5}$$

where $\bar{y}_p$ = the predicted mean proportion;
σ^2 = obtained from the mean squared residuals across 20 catches by 200 analyses; and
σ_k^2 = the estimated variance of k across 20 catches by 200 analyses.

All p and y values are presented as percentages in results.

Abundance estimates The effect of taking different size subsamples, on estimating the total number of a given species in a catch, was determined by using a running mean (of the estimate of abundance) calculated from the equation

$$s = \left|(n / p - TotNum) / TotNum\right|, \tag{6}$$

where s = the absolute proportion of sampling error;
n = the observed number of that species after p proportion (by weight) of the catch has been sorted; and
$TotNum$ = the total number of individuals of that species in the whole catch.

The values for s are truncated at 1 for ease of presenting results.

We used the following statistical model in which s is subtracted from 1 in order to correspond to the equation used for species composition:

$$1 - s = p^k + \varepsilon, \tag{7}$$

where $1-s$ is fixed at 1 when $p = 1$, and the var(ε) = $(1-p)\,\sigma^2$ to ensure that there is no sampling error when the entire catch has been sorted.

To obtain estimates of σ^2, we fitted the following model:

$$(1-s)/\sqrt{(1-p)} = p^k/\sqrt{(1-p)} + \varepsilon/\sqrt{(1-p)}, \tag{8}$$

where the errors for this model have homogeneous variance structure. The variance of a predicted (1–s) is given by an equation similar to Equation 5 (for species composition):

$$\sigma_m^2 = \left(\log(p)(1-s)\sigma_k\right)^2 + (1-p)\sigma^2. \tag{9}$$

To examine the accuracy in recording the abundance of all the species in a catch, we modeled the order (200 times) in which subsamples were taken (as described above for catch composition estimates). For each (species by trawl) case, i.e. where a species was recorded at any level of abundance, we calculated the sampling error s for ranges of p from 0.1 to 0.9. We grouped all the (species by trawl) cases of different levels of abundance into eight categories for these analyses. They were <1; 1 to <2; 2 to <3; 3 to <4; 4 to <5; 5 to <10; 10 to <50 and 50 or more per 10-kg subsample respectively.

The SAS procedure NLIN was used to fit the power curve for catch composition, as well as the separate power curves for sampling error for the different abundance classes.

Results

General results

Catches ranged in size from 71 to 445 kg, with an average of 117 species per trawl (84 fish and 33 invertebrate species). A total of 140,253 fish and invertebrates were recorded from 323 subsamples taken from the 20 prawn trawl catches that were sorted entirely (Table 1). Subsamples weighed, on average, 11.2 kg and contained 434 individuals (or 389 individuals per standardized (std) 10-kg subsample). We identified a total of 276 fish and 141 invertebrate species.

A total of 69.3% (1617 out of 2333) of the (species × trawl) cases of relative abundance were recorded at ratios of less than one individual per std 10-kg subsample (or less than one in every 389 individuals), when averaged over all 20 catches; they were classed as "rare" (Fig 2). A further 19% (442 out of 2333) of the (species × trawl) cases of relative abundance were recorded at ratios that fell between one individual per std 10-kg subsample and less than five individuals per 10-kg subsample (or between one in 389 and less than five in 389 individuals), when averaged over all 20 catches; they were classed as "common." The remaining 11.7% (274 out of 2333) of the (species × trawl) cases of relative abundance were recorded at ratios of five or more individuals per std 10-kg subsample (or five or more in every 389 individuals), when averaged over all 20 catches); they were classed as "abundant" (Fig 2).

Catch composition

The number of species recorded increased as the weight of sorted catch increased in 19 of the 20 catches. This relationship appeared to reach an asymptote in the remaining large catch of 445 kg (Fig 3, A and B).

After 10% of all 20 catches were sorted, the cumulative percentage of species recorded ranged from 31% (in the 315 kg catch) to 78% (in the 182 kg catch) (Table 2). To detect 80% of the species present in a single catch, from 20% to 70% of the catch had to be sorted.

Simulation modelling showed that sorting 10% of catch weight detects (on average) 50% of the species present, with the confidence interval ranging from 44% to 57% (Fig 4). Sorting 50% of the catch was necessary to detect 80% of the species present.

Abundance estimates The simulation model showed that the mean sampling error curves (for the eight abundance categories) decreased as increasing percentages of the catch had been sorted (Fig 5). After 10% of the weight of

Table 2

The percentage of species recorded as more of the catch is sorted (10% increments). Percentages were calculated separately for 20 trawl catches that were completely sorted. The demarcation line denotes where 80% or more of the species in each catch were recorded.

Region	Catch weight (kg)	Number of species	Cumulative % of species recorded (in 10% Wt increments)								
			10%	20%	30%	40%	50%	60%	70%	80%	90%
North of Mornington Island	71	90	51	62	70	76	81	86	90	94	97
North of Groote Eylandt	85	94	49	60	69	75	81	85	89	93	97
East of Mornington Island	87	122	56	66	74	79	84	88	91	95	97
Torres Straits	94	95	60	70	76	81	86	89	92	95	98
North of Mornington Island	100	111	56	66	74	79	84	88	91	95	97
North of Groote Eylandt	147	96	50	61	69	76	81	86	90	93	97
East of Vanderlin Islands	156	90	39	52	61	69	75	81	86	91	96
North of Groote Eylandt	158	132	65	74	80	84	88	91	94	96	98
South of Groote Eylandt	165	89	51	63	71	77	82	86	90	94	97
Weipa	166	128	54	65	73	78	83	87	91	94	97
North of Groote Eylandt	169	159	57	68	75	80	85	88	92	95	98
Melville	170	90	46	58	67	73	79	84	89	93	97
West of Mornington Island	174	121	52	63	71	77	82	86	90	94	97
North of Mornington Island	182	137	78	84	88	91	93	95	96	98	99
North of Groote Eylandt	189	139	61	71	77	82	86	90	93	95	98
North of Mornington Island	194	136	67	76	81	85	89	92	94	96	98
West of Mornington Island	269	111	52	63	70	77	82	86	90	94	97
East of Mornington Island	274	108	40	53	62	69	76	82	87	92	96
South of Groote Eylandt	315	130	31	44	54	63	70	77	84	89	95
North of Mornington Island	445	166	61	71	77	82	86	90	93	95	98

catches had been sorted, only two abundance categories (from ≥10 to <50, and ≥50 per subsample) had mean sampling error rates below 25%.

For the "rare" species (<one per subsample), the gradient of the mean sampling error curve was close to constant (Fig 6). The 95% upper confidence interval was over 100% until more than 40% of the catches had been sorted. Even when 90% of the catches had been sorted, the mean sampling error was just below 10%, and the 95% upper confidence interval remained above 25%.

For the "abundant" species (five or more per subsample), the mean sampling error curve started just below 25% after 10% of catches had been sorted, and fell below 10% when more than 40% of the catches had been sorted (Fig 6). The 95% confidence interval did not fall below 25% until 50% of catches had been sorted.

Discussion

This study shows that a large subsample is required to accurately represent the species composition of a large multispecies catch from

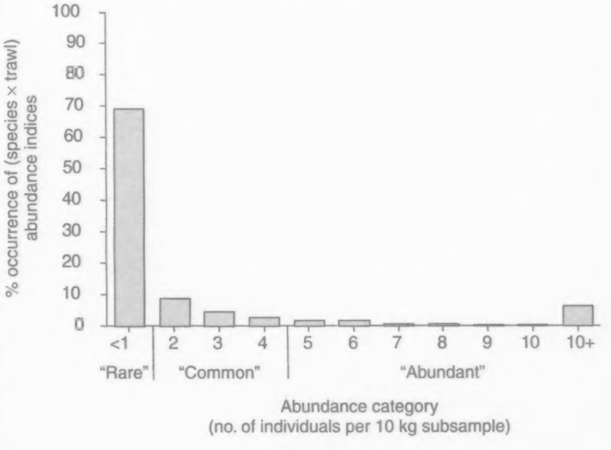


Figure 2

The percentage frequency of occurrence of 2333 cases of (species × trawl) relative abundance for bycatch species recorded from 20 trawl catches. The cases are grouped into 11 categories of abundance indices based on the average number of a species recorded per 10-kg subsample of catch.

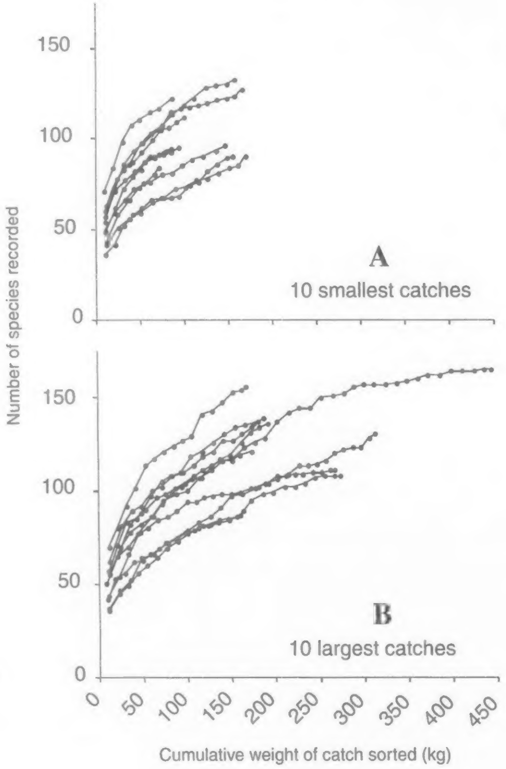


Figure 3

The cumulative numbers of bycatch species recorded as the weight of catch analysed increased for (**A**) the 10 smallest and (**B**) the 10 largest catches that were entirely sorted.

the Northern Prawn Fishery (NPF). As more of the catch is sorted, more new species are encountered. On average, 50% of the catch weight needs to be sorted to record 80% of the species in a single catch. Our data suggest that taking subsamples of between 10% and 30% of catch weight can result in highly variable percentages (from 31% to 88%) of the total species in the catch. When estimating the relative abundance of individual species within a catch, subsampling small percentages of catch weight (around 10%) results in a sampling error around 80% for "rare" species, and around 25% for "abundant" species.

The sampling error (when estimating the relative abundance of a species in a trawl) is a function of the total number of bycatch individuals caught in that trawl. For example, 10 individuals of species "*X*" may occur in one trawl, at a ratio of one in every 100 bycatch individuals. In the very next trawl, 10 individuals of species "*X*" may occur at a ratio of only one in every 1000 bycatch individuals because other "abundant" species have swamped its occurrence ratio. As a consequence, the sampling error for the same number of individuals of species "*X*" varies greatly between trawls.

There are two sources of within-trawl variation to take into account when calculating catch rates for individual species. The first is due to changes in catchability at the trawl-species interface (either on the sea floor or in the water column). The second is due to on-deck subsampling techniques. This study has been able to allocate percentages of sampling error based on the occurrence rate of a species of interest within individual catches. To do this, we calculated the average occurrence ratios for the different categories of relative abundance used in this study. For example, "rare" species occurred at a rate of less than one individual in every 389 bycatch individuals; "abundant" species occurred at a rate of five or more individuals in every 389 bycatch individuals. These ratios can now be applied to species of interest recorded in other trawl catches and the average sampling errors can be calculated.

Monitoring catch rates of a suite of "indicator" bycatch species is a possible future option for measuring the health of nontarget species in trawl fisheries such as the NPF. Stobutzki et al. (2001) identified a suite of small NPF fish species that are more likely to be impacted by trawling.

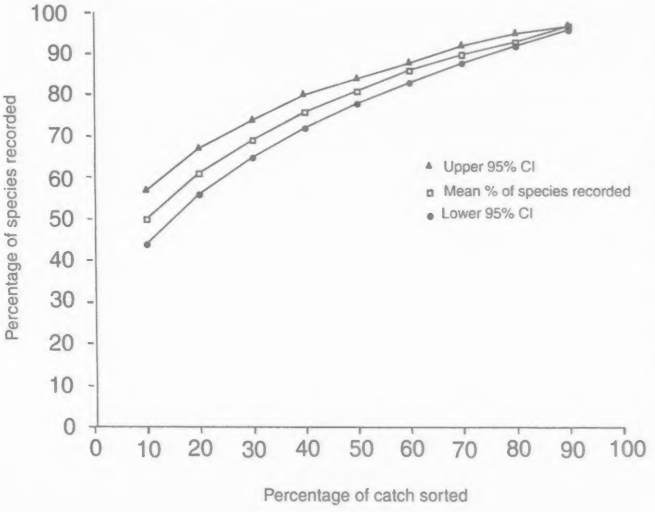


Figure 4

The mean percentage (and 95% CI) of species recorded as increasing percentages of catch weight were sorted. Curves were generated from 200 random selections of the numbered order in which subsamples were collected from each catch.

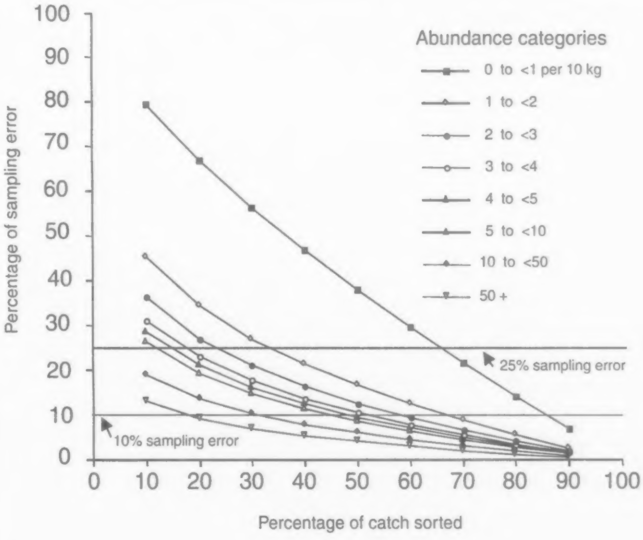


Figure 5

The mean percentage of sampling error calculated when estimating the relative abundance of bycatch species grouped into eight categories of relative abundance. Curves were generated from 200 random selections of the numbered order in which subsamples were collected from each catch.

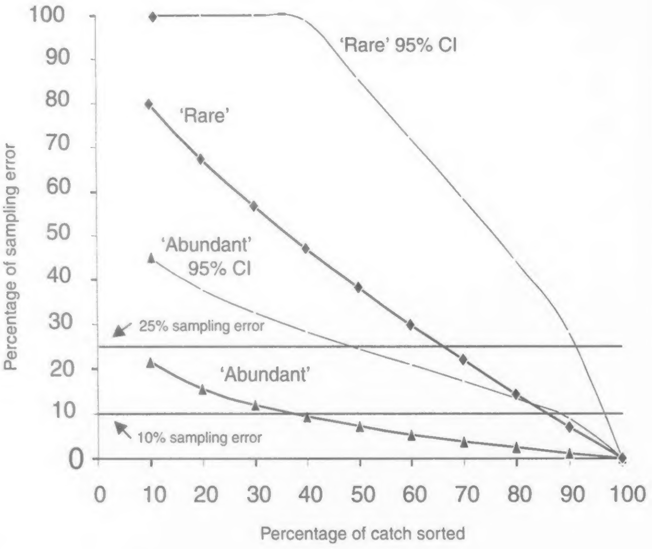


Figure 6

The mean percentage of sampling error calculated when estimating the relative abundance of the "rare" (<1 individual per 10-kg subsample) and the "abundant" bycatch species (5 or more individuals per 10-kg subsample). Curves were generated from 200 random selections of the numbered order in which subsamples were collected from each catch.

The catch rates of many of these most-at-risk species are extremely low. Our data suggest that extremely high sampling errors will be incurred if monitoring catch rates of species on this list is undertaken. These errors must be incorporated into any models that assess the impact of trawling on populations of these species.

The range of data in the present study includes many of the sources of variation likely to be encountered in setting up a wide-ranging bycatch monitoring program. The results we present are a valuable guide to the accuracy of subsampling. In particular, this study emphasizes the value of collecting large subsamples, especially when one is restricted to representing the bycatch of an area by only one or a few trawls. This problem is common for research cruises when many regions need to be sampled in a short time (e.g. Blaber et al., 1994), and is also a common problem for observers on commercial fishing vessels where catch sampling is often restricted by the nature of commercial practices. Because there is a high level of sampling error when estimating the abundances for "rare" species, reliable estimates will require either taking large subsamples or sorting entire catches.

The size of some catches in our study may be larger than those of many other tropical prawn trawl fisheries in Australia and overseas. However, the data in the matrix on the range of cumulative species (per proportion of catch sorted, Table 2) will allow managers of other trawl fisheries to better understand the implications and likely accuracy of bycatch sampling programs.

Acknowledgments

We thank S. Cook, M. Farmer, C. Liron, D. Milton, J. Salini, I. Stobutzki, T. Wassenberg, G. Fry, and others for their valued help in sorting the catches both on board the RV *Southern Surveyor* and in the Cleveland laboratory. We also thank D. McKay, the skipper of FV *Apolloair,* and P. Hoschke, the skipper of FV *Ventura,* and their respective crews, for their help in collecting commercial trawl samples. We also thank J. Bishop, S. Blaber, B. Hill, D. Milton, and V. Mawson for their valuable comments on the manuscript. This work was undertaken with the support of FRDC grant no 96/257

Literature citations

Andrew, N. L., and B. D. Mapstone.
1987. Sampling and the description of spatial patterns in marine ecology. Oceanogr. Mar. Biol. Annu. Rev. 25:39–90.

Blaber, S. J. M., D. T. Brewer, and A. N. Harris.
1994. Distribution, biomass and community structure of demersal fishes of the Gulf of Carpentaria, Australia. Aust. J. Mar. Freshw. Res. 45:375–396.

Blaber, S. J. M., D. T. Brewer, J. P. Salini, and J. Kerr.
1990. Biomasses, catch rates and abundances of demersal fishes, particularly predators of prawns, in a tropical bay in the Gulf of Carpentaria, Australia. Mar. Biol. 107: 397–408.

Brewer, D., N. Rawlinson, S. Eayrs, and C. Burridge.
1998. An assessment of bycatch reduction devices in a tropical Australian prawn trawl fishery. Fish. Res. 720:1–21.

FAO (Food and Agriculture Organization of the United Nations).
1995. Code of conduct for responsible fisheries, 41 p. FAO, Rome, Italy.

Harris, A. N., and I. R. Poiner.
1991. Changes in species composition of demersal fish fauna of the Southeast Gulf of Carpentaria, Australia, after 20 years of fishing. Mar. Biol. 111:503–519.

Heales, D. S., D. T. Brewer, and Y.-G. Wang.
2000. Subsampling multi-species trawl catches from tropical northern Australia: does it matter which part of the catch is sampled? Fish. Res. 48:117–126.

Martin, T. J., D. T. Brewer, and S. J. M. Blaber.
1995. Factors affecting distribution and abundance of small demersal fishes in the Gulf of Carpentaria. Mar. Freshw. Res. 46:909–920.

Pender, P. J., R. S. Willing, and B. Cann.
1992. NPF bycatch a valuable resource? Aust. Fish. 51(2): 30–31.

Poiner, I. R., and A. N. M. Harris.
1986. The effect of commercial prawn trawling on the demersal fish communities of the south-eastern Gulf of Carpentaria. *In* Torres Strait fisheries seminar, Port Moresby, 11–14 February 1985 (A. K. Haines, G. C. Williams, and D. Coates, eds.), p. 239–261. Australian Government Publishing Service, Canberra, Australia.

Polet, H., and F. Redant.
1999. Effect of population structure, sampling strategy and sample size on the estimates of selection parameters for shrimp (*Crangon crangon*) trawls. Fish. Res. 40:213–225.

Ramm, D. C., P. J. Pender, R. S. Willing, and R. C. Buckworth.
1990. Large scale spatial patterns of abundance within the assemblage of fish caught by prawn trawlers from Northern Australian waters. Aust. J. Mar. Freshw. Res. 41:79–95.

Snedecor, G. W., and W. G. Cochran.
1980. Statistical methods, 7th ed., 593 p. Iowa State Univ. Press, Ames, IA.

Stobutzki, I., S. Blaber, D. Brewer, G. Fry, D. Heales, M. Miller, D. Milton, J. Salini, T. Van der Velde, T. Wassenberg, P. Jones, Y-G. Wang, M. Dredge, T. Courtney, K. Chilcott, and S. Eayrs.
2000. Ecological sustainability of bycatch and biodiversity in prawn trawl fisheries. Rep. 96/257, 512 p. Fisheries Research Development Corp., Deakin West, Australia.

Stobutzki, I., M. Miller, and D. Brewer.
2001. Sustainability of fishery bycatch: a process for assessing highly diverse and numerous bycatch. Environ. Conserv. 28(2):167–181.

Tamsett, D., G. Janacek, and M. Emberton.
1999. A comparison of methods for onboard sampling of discards in commercial fishing. Fish. Res. 42:127–135.

Taylor, B., and D. Die, eds.
1999. Northern prawn fishery assessment report, 1997 and 1998. AFMA (Australian Fisheries Management Authority), Canberra, Australia.

Van Ark, H., and R. Meiswinkel.
1992. Subsampling of large light trap catches of *Culcoides* (Diptera: Ceratopogonidae). Onderstepoort J. Vet. Res. 59: 183–189.

Vinson, M. R.
1996. Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. J. North Am. Benthol. Soc. 15(3):392–399.

Walsh, C. J.
1997. A multivariate method for determining optimal subsample size in the analysis of macroinvertebrate samples. Mar. Freshw. Res. 48:241–248.

Wassenberg, T. J., C. Y. Burridge, M. Connell, and N. Gribble.
1998. A validation of short-duration scientific tows as a representation of long commercial-length tows: comparing the catch rates, size composition and species composition of prawn trawler bycatch in the far northern Great Barrier Reef, Australia. Fish. Res. 36:35–46.

Williams, G. C.
1985. The Torres Straits prawn fishery. *In* Torres Strait fisheries seminar, Port Moresby, 11–14 February 1985 (A. K. Haines, G. C. Williams, D. Coates, eds.), p. 233–238. Australian Government Publishing Service, Canberra, Australia.

Age and growth of blue rockfish (*Sebastes mystinus*) from central and northern California

Thomas E. Laidig

Donald E. Pearson

Southwest Fisheries Science Center
National Marine Fisheries Service
110 Shaffer Rd.
Santa Cruz, California 95060
E-mail: tom.laidig@noaa.gov

Lorraine L. Sinclair

Pacific States Marine Fisheries Commission
411 Burgess Drive
Menlo Park, California 94025

Abstract—Otoliths from blue rockfish (*Sebastes mystinus*), were aged by using a combination of surface and break-and-burn methods. The samples were collected between 1978 and 1998 off central and northern California. Annual growth increments in the otoliths were validated by using edge analysis for females up to age 23 and for males to age 25. The first annual growth increment was identified by comparing the diameter of the otolith from fish known to be one year old collected in May (when translucent zone formation was completed) to the mean diameter of the first translucent zone in the otoliths from older fish. Our estimated maximum ages of 44 years for males and 41 years for females were much older than those reported in previous studies. Von Bertalanffy growth models were developed for each sex. Females grew faster and reached larger maximum length than males. The growth models were similar to those generated in other studies of this species in southern and central California. Fish from northern and central California had similar maximum sizes, maximum ages, and growth model parameters.

Accurate information on age and growth is critical for reliable assessments and effective management of fish stocks. Most assessments of west coast groundfish stocks use age-based models (PFMC, 2001). It is important to obtain reliable ages for maturity schedules, age-specific fecundity, and age-specific selectivity, as well as estimates of aging accuracy, in order to correctly estimate biomass and acceptable biological catch numbers for these assessments. Inaccurate age estimates can lead to over-harvesting or denial of fishing opportunities.

In the present study, we examine age and growth of blue rockfish (*Sebastes mystinus*). The blue rockfish is a schooling species that occurs from Sitka Strait (southeast Alaska) to northern Baja California (Love et al., 2002). They reach a maximum size of 508 mm fork length (FL). Blue rockfish are frequent inhabitants of nearshore rocky reefs, and are commonly found from the surface to about 90 m water depth. Blue rockfish comprise a major fraction of the recreational fishery off California (Miller and Geibel, 1973; Karpov et al., 1995) but are less common in the commercial fishery. In 1994, blue rockfish landings off California totaled 172 metric tons (t) from recreational fisheries and only 68 t from commercial fisheries (Rogers et al., 1996).

Age structure of blue rockfish has been determined previously by using length-frequency analyses, tag-and-recapture studies, scales, and whole otoliths (Wales, 1952; Miller and Geibel, 1973; McClure, 1982; MacGregor, 1983; Karpov et al., 1995). We estimated age by examining the surface of whole otoliths and broken-and-burnt cross sections of otoliths. Aging the surface of whole otoliths is only effective for young rockfish (Six and Horton, 1977; Kimura et al., 1979; Chilton and Beamish, 1982). The break-and-burn technique (Chilton and Beamish, 1982) is used widely for age determinations of west coast groundfish (including Dover sole [*Microstomus pacificus*], sablefish [*Anoplopoma fimbria*], and numerous species of rockfishes).

We used edge analysis to verify the annual periodicity of growth increments in the otoliths of blue rockfish. Edge analysis and marginal increment analysis have been used to validate annual growth increments in numerous species. In recent studies, Crabtree and Bullock (1998) validated the first seven annual growth increments in black grouper, and Brown and Sumpton (1998) validated the ages of the redthroat emperor off Australia. The procedure has been used to validate annual growth increments in many rockfish species, including redfish, (Mayo et al., 1981), yellowtail (Kimura et al., 1979), shortbelly (Pearson et al., 1991), widow (Pearson, 1996), gopher, and kelp rockfish (Lea et al., 1999).

Manuscript approved for publication 17 April 2003 by Scientific Editor.

Manuscript received 26 June 2003 at NMFS Scientific Publications Office.

Fish Bull. 101:800–808.

Methods

Rockfish sampling

Otoliths of blue rockfish were obtained from 1) the recreational catch of commercial passenger fishing vessels (CPFV); 2) the catches of midwater trawls deployed from a research vessel; and 3) specimens speared by researchers equipped with SCUBA (Table 1). CPFV landings were available from Monterey to Bodega Bay, California, from 1978 to 1998. All fish were caught at depths deeper than 20 m; total length (TL) and sex were recorded for each specimen, and otoliths were removed. Pelagic young-of-the-year blue rockfish were caught in a 13 × 13 m midwater trawl deployed periodically from the RV *David Starr Jordan* off the central California coast from Monterey to Marin counties during 1988–93 at depths of 5–30 m. In the laboratory, each specimen was measured (standard length [SL]) and otoliths were removed and attached to microscope slides for later examination. Specimens were taken with spears from 1988 to 1998 in water depths of 1–20 m off Sonoma and Mendocino counties (Table 1). Some cohorts were sampled throughout their first year after first settlement, thus providing specimens of known age. We measured each specimen, determined sex, and removed otoliths. All fish lengths (either TL or SL) were standardized to FL for comparisons (by using equations from Echeverria and Lenarz, 1984).

Otolith examination

Ages were estimated by counting the number of translucent zones from the surface of whole otoliths for young blue rockfish (less than 5 years of age) and by using the break-and-burn method (Chilton and Beamish, 1982) for fish greater than 5 years old. Whole otoliths were viewed through a dissecting microscope at 20–40× magnification with reflected light. For the break-and-burn method, whole otoliths were broken in half through the core, and one broken section was burned and viewed through a dissecting microscope at 20–40× magnification. Two readers determined ages independently by counting the number of translucent zones observed in both whole and broken-and-burnt otoliths. The precision of age estimates was compared by using the average percent error (APE; Beamish and Fournier, 1981). Otolith diameter was measured from the dorsal to the ventral edge through the core of the otolith. The diameter of the otolith at each presumed annual growth increment was measured from a video image of the cross section of a broken section of the otolith from the dorsal edge to the ventral edge of the translucent zone along a transverse axis through the core.

Validation of growth increments

Validation of growth increments as being produced annually was conducted in four parts. First, we conducted an edge analysis (Pearson, 1996) to determine if only one translucent zone formed along the edge of the otolith during a year. We identified the leading growth edge as either "opaque" or "translucent" on otoliths collected throughout the year to determine when the translucent zone was formed. An examination of edge analysis data was conducted on each age class from 1 to 44 years to determine if only one opaque and one translucent zone formed annually, and if they had formed, then these ages were considered validated.

Second, to identify the first annual growth increment, the average diameter of otoliths from known age one-year-old fish was compared to the average diameter of the first translucent zone from older fish. We determined the length of one-year-old fish at the time of translucent zone completion (as determined by the edge analysis) by plotting fish length against Julian date of capture and then determined the best model that described this relationship.

Third, we determined the predicted otolith diameter of a fish at the time of translucent zone completion. For this analysis, we used fish lengths from young-of-the-year (YOY; both pelagic and settled) and one-year-old blue rockfish for which ages were known by following a cohort through time. Fish length was plotted against total otolith diameter, and the best model for this relationship was determined. Using this relationship and the size of a one-year-old fish (established from the previous model), we calculated otolith diameter for one-year-old fish.

Fourth, mean diameters of the first, second, and third translucent zone of all otoliths from older fishes were measured. The diameters of these three translucent zones were compared to each other by using a Student's t test. If the first translucent zone corresponded to the diameter of an otolith at the time of completion of the first translucent zone and the first translucent zone was significantly different from the second and third zones, then the assumption that the first translucent zone was equivalent to the first annual growth increment was considered validated.

Table 1

Number of specimens collected by year for blue rockfish (*Sebastes mystinus*) from three different sampling methods.

Year	Recreational catch	Diver spears	Midwater trawls
1978	12		
1980	64		
1981	214		
1982	215		
1985	31		
1986	77		
1988		109	35
1989		217	11
1990		206	38
1991		157	34
1992		122	7
1993		190	8
1994		138	
1995		18	
1997	15	43	
1998	27	45	

Growth

A von Bertalanffy growth curve was fitted to the fish length and age data. The form of the equation was

$$L_t = L_\infty (1 - e^{-k(t - t_0)}),$$

where L_t = fish length (mm FL) at age t;
L_∞ = maximum fish length (mm FL),
k = growth completion rate (per year);
t = age (years); and
t_0 = theoretical age (years) when the fish was length zero.

Parameters for the growth curve were calculated iteratively by using the method described by Schnute (1981). Growth models were developed separately for males and females to account for possible sex-specific growth rates (Echeverria, 1986). Growth curves were fitted for the entire sampling area and separately for the northern and southern areas to examine potential latitudinal trends in growth and by mode of collection. Growth curves were compared by using the extra sum of squares principle (Draper and Smith, 1981; Ratkowsky, 1983; Pearson and Hightower, 1991).

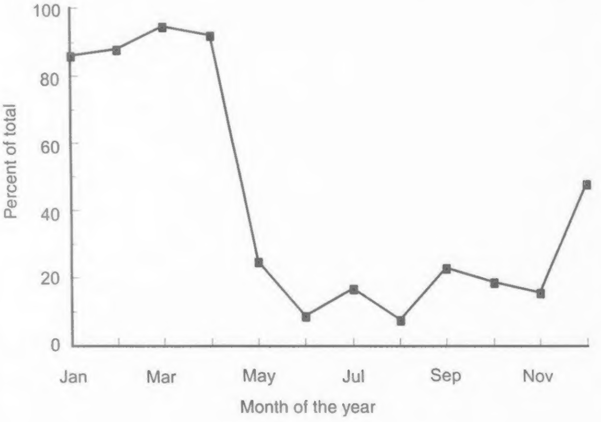


Figure 1

Percentage of blue rockfish (*Sebastes mystinus*) having a translucent zone along the otolith edge in each month (n=927).

Results

A total of 1980 blue rockfish were examined; 655 of these fish were caught by hook-and-line from CPFV, 133 pelagic juveniles were collected in midwater trawls offshore, and 1245 fish were speared. Maximum size of fish from the CPFVs was 365 mm FL for males and 444 mm FL for females. Maximum size of speared fishes was 360 mm FL for males and 412 mm FL for females. The oldest fish from the CPFVs were a 44-year-old male and a 40-year-old female, and the oldest speared fish were a 39-year-old male and a 41-year-old female. There was a 5.6% APE between readers. When ages were not in agreement, the readers would discuss the differences and if no consensus could be reached, the suspect otoliths were discarded.

Validation of growth increments

A subset (out of 1900 otoliths used for aging) of 927 (603 from CPFV and 324 from spearing) otoliths ranging from 1 to 44 growth increments was examined for edge analysis. Formation of the translucent zone followed a seasonal pattern for all otoliths combined (Fig. 1). A translucent zone developed from December through April, followed by an opaque zone that developed from May to November. Translucent zones in over 70% of the otoliths were completely formed by 1 May (or 120 Julian days), and formation of the opaque zone was complete by January in over 80% of the otoliths. From these results, we concluded that only one translucent and one opaque zone formed during a calendar year. Because only one translucent zone was shown to form each year, the annual periodicity of these zones was established. To complete the validation at each age, an edge analysis should be conducted for each age class individually (Campana, 2001). There were enough samples to conduct an edge analysis for females up to age 23 and males to age 25, and, in all instances, only one opaque and one translucent zone formed annually. Therefore, female blue rockfish were validated up to 23 years and males up to 25 years.

The length of a fish at the conclusion of translucent zone formation (determined from the edge analysis) was calculated. The first translucent zone was calculated to be complete on 1 May, one year after the assumed parturition on 1 January, or 365 Julian days + 120 Julian days = 485 days from parturition. The relationship between date of capture and FL was described with the linear equation: $FL = 0.16 \times$ (date of capture) + 30.9 (n=99, r^2=0.91; Fig. 2). From this equation, a fish would be 108.5 mm FL at the time of completion of the first translucent zone (1 May, one year after parturition).

The relationship between fish length and otolith diameter (Fig. 3) was best described by the linear equation *otolith diameter* (mm) = 0.02 × (mm FL) − 0.02 (n=198, r^2=0.95). From this equation and a fish length of 108.5 mm FL at the time of the first translucent zone completion, an otolith diameter of 2.19 mm was the estimated size of the predicted translucent zone.

Diameters of the first and second translucent zones (n=509, df=508, $P<0.001$) and the second and third translucent zones (n=151, df=150, $P<0.05$) differed significantly. From a comparison of first, second, and third translucent zone diameters in the otoliths of all blue rockfish against fish length, the average observed diameter for the first translucent zone was 2.17 mm (Fig. 4), compared to the estimated diameter of 2.19 mm (from the above equation), the average observed diameter of the second zone was

3.00 mm, and the average observed diameter of the third zone was 3.67 mm (Fig. 4).

Growth

Growth between the sexes was significantly different ($P<0.05$); females grew faster and reached a larger maximum size than did males (Fig. 5). The von Bertalanffy growth model parameters for females were $t_0 = -1.34$ years, $k = 0.149$/year, and $L_\infty = 400.16$ mm FL and for males were $t_0 = -0.95$ years, $k = 0.195$/year, and $L_\infty = 329.41$ mm FL.

Growth models for both sexes were not significantly different ($P>0.05$) between the southern CPFV and northern speared samples (Fig. 6). The models representing males were virtually identical, with parameters for the CPFV model of $t_0 = -0.94$ years, $k = 0.195$/year, and $L_\infty = 331.66$ mm FL, and for the speared model of $t_0 = -0.99$ years, $k = 0.194$/year, and $L_\infty = 323.14$ mm FL. There was no significant difference between females; $t_0 = -1.94$ years, $k = 0.107$/year, and $L_\infty = 430.74$ mm FL for the CPFV samples and $t_0 = -1.14$ years, $k = 0.166$/year and $L_\infty = 393.34$ mm FL for the speared. Females from CPFV were larger at ages after 15 years than those that had been speared.

Discussion

We estimated the age (using the break-and-burn technique) of blue rockfish to be greater than that reported in earlier studies. Aging the scales of blue rockfish, Miller and Geibel (1973) reported maximum ages of 24 and 17 years for females and males, respectively, whereas the oldest of either sex reported by MacGregor (1983) was only 13 years. Based on modal progression of length distributions, the estimate of the oldest individuals of either sex calculated by Karpov et al. (1995) was 17 years. In a study of blue rockfish off Newport, Oregon, McClure (1982) examined otolith surfaces and determined that the oldest female was 16 years, and the oldest male was only 12 years. In aging males to 44 years and females to 41 years, our study more than doubled the recorded maximum ages for blue rockfish, demonstrating the value of the break-and-burn section method for accurate age determination.

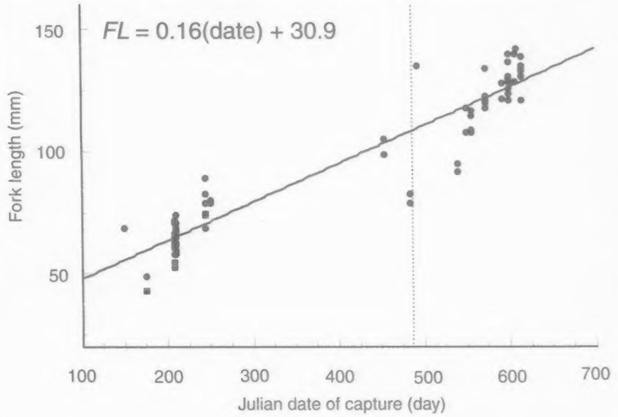


Figure 2

Growth of blue rockfish (*Sebastes mystinus*) collected nearshore during their first 1.5 years. Solid line is the fitted linear growth model (n=99; r^2=0.91). Vertical line represents 1 May, one year after the parturition date of 1 January (i.e. 365 +120=485 days).

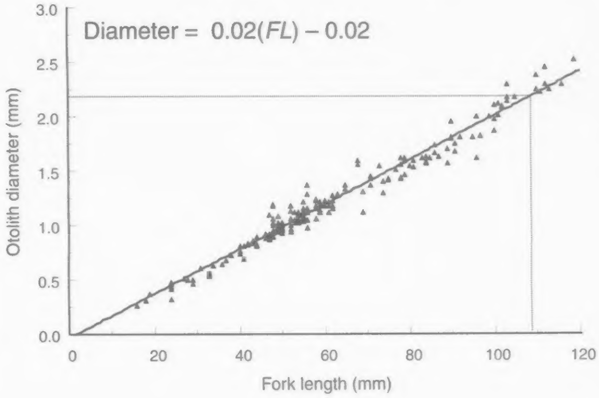


Figure 3

Change in otolith diameter with fish length for young-of-the-year and one-year-old blue rockfish (*Sebastes mystinus*). Solid line is the fitted linear model (n=198; r^2=0.95). Dashed line represents an estimated total otolith diameter of 2.19 mm for a fish of 108.5 mm FL (i.e. fish length at time of translucent zone completion).

Age data were validated by using an edge analysis and the first translucent zone was validated as corresponding to the first annual growth increment. Campana (2001) pointed out that there are problems in using edge analysis as a validation tool. Specifically the extension of younger, validated ages to older, nonvalidated ages. In our study, we validated ages up to 23 years for females and up to 25 years for males; ages of older fish could not be positively validated. Therefore, caution must be taken when using the older ages.

The growth rates of blue rockfish in our study were similar to those estimated by others in California, but slower than conspecifics off Oregon (Fig. 7). MacGregor (1983) examined blue rockfish from southern California and determined the combined male and female growth

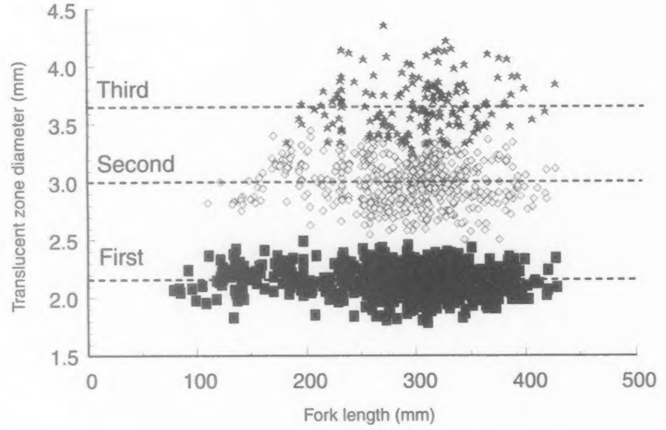


Figure 4

Diameter of the first, second, and third translucent zones in otoliths from blue rockfish (*Sebastes mystinus*) at various lengths. Dashed lines represent the mean diameters.

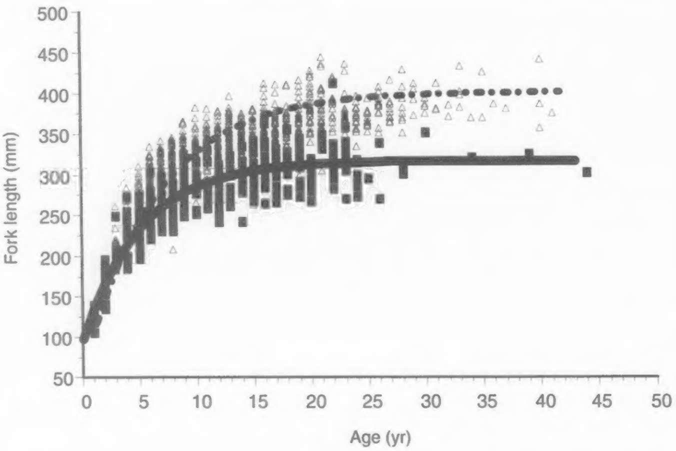


Figure 5

Von Bertalanffy growth models for all male (solid line) and female (dashed line) blue rockfish (*Sebastes mystinus*). (n=348 for males and n=1348 for females.)

rate and calculated k (instantaneous growth rate) to range from 0.13–0.16/years, which was comparable to k in our study (0.2/year for males and 0.15/years for females; mean k=0.17/year). Karpov et al. (1995) calculated k for the combined male and female growth rate from modal progressions studies to be 0.12/year. This also was similar but less than the k from our present study. On the other hand, McClure (1982) estimated a much faster growth rate for blue rockfish off Oregon, with a k for males of 0.23/years and for females of 0.31/year. Although the Oregon fish were larger at age (Fig. 7), maximum sizes from Oregon and California were similar; the largest specimen from Oregon was 460 mm FL (McClure, 1982) and the largest individual from our study was 444 mm FL.

The difference in growth between studies may be attributed to a temporal difference in the collection of fish. Two thermal regime shifts have occurred in the Pacific Ocean over the past 25 years; one in 1977 and the other in 1989 (Hare and Mantua, 2000). The samples from our study came from two different thermal regimes, but the growth curves were not statistically different (Table 1). Therefore, these regimes did not appear to effect the growth of adult fish. Out of the four other surveys mentioned above, three came from one of these two regimes, and the fourth, Miller and Geibel (1973), came from an earlier regime. If there were any effects from the three different thermal regimes, it would seem clear that these differences would show up between samples from such varied regimes. However, the only study with different measures of growth was that from Oregon (McClure, 1982), with samples that were collected during the same regime as two of the other studies (MacGregor, 1983; and the present study). Therefore, thermal regime alone does not seem to have a major impact on the growth of blue rockfish, although further analysis is needed to confirm this point.

These differences in growth parameters between fish from California and Oregon may be attributed to differences in aging methods. Wilson and Boehlert (1990) found that estimates of growth based on aging of otolith surfaces were higher for *Sebastes pinniger*, but were similar to growth rates estimated from otolith sections for *S. diploproa*. The ages of *S. alutus* determined from otolith surfaces had poor correlation with ages from otolith cross-sections for fish older than 17 years, but there was close agreement for younger fish (Stanley and Melteff, 1987). Reading ages from the surface of an otolith may underestimate the age of a rockfish (Munk, 2001) and thus result in greater size-at-age and growth rate estimates. However, aging methods may not be the only factor influencing the growth discrepancies. Miller and Geibel (1973) and MacGregor (1983) both used scales to age blue rockfish (which also can underestimate the age of fish [Beamish and MacFarlane, 1987]), and, yet, their growth models more closely approximated the model produced by our study.

Faster growth estimated for blue rockfish off Oregon may reflect a latitudinal difference in growth. Fraidenburg (1980) examined length and age composition of *Sebastes flavidus* and reported evidence of a north-to-south cline of decreasing size-at-age. Pearson and Hightower (1991) studied *S. entomelas* and noted smaller *k* values and larger average maximum lengths with increasing latitude. Boehlert and Kappenman (1980) reported faster growth in the north for *S. diplopora* and no difference in growth with latitude for *S. pinniger*. They postulated that because the fish live demersally on the continental shelf, latitudinal variation in environmental factors may be insufficient to explain the difference in growth rates and that differential exploitation by the fishery may be a possible influence on growth. Blue rockfish live at relatively shallow depths where environmental and biological factors may have a greater influence on their populations.

Although blue rockfish display a possible latitudinal trend in growth rate between California and Oregon, within California no latitudinal trend in growth rates was observed. Specimens from both the southern CPFV sample and the northern speared sample areas had translucent zone completion by 1 May, which was consistent with the findings of Miller and Geibel (1973) using ages from scales. Individual fish in our study also had similar maximum ages and maximum fish lengths in the north and south areas. No latitudinal trend in growth rates was observed over the 280 km between the centers of the two sampling areas. Although growth rates varied throughout their study area from Half Moon Bay in the north to Morro Bay in the south, Miller and Geibel (1973) likewise observed no latitudinal trend in growth for blue rockfish.

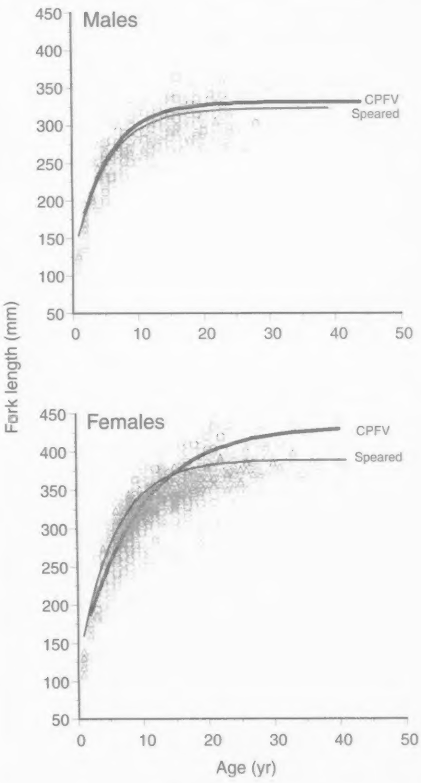


Figure 6

Von Bertalanffy growth models for male and female blue rockfish (*Sebastes mystinus*) from the CPFV (thick line) and speared samples (narrow line). Individual data points are plotted for blue rockfish from CPFV (open circle) and speared samples (open triangles).

Blue rockfish have average maximum ages and growth rates when compared to other rockfish species. Maximum ages for rockfishes (*Sebastes* spp.) range from 12 years for the relatively small calico rockfish to 205 years for rougheye rockfish, one of the largest species (Cailliet et al., 2002; Love et al., 2002). According to Love and Johnson (1998), of the 38 species most accurately aged, most lived to more than 40 years. Love et al. (1990) found growth rates for three species that share the blue rockfish habitat (black, k=0.12–0.21/year; yellowtail, k=0.16–0.20/year; and widow rockfish, k=0.14–0.22/year) to be similar to that for blue rockfish (k=0.17 years). Mean k values for rockfish varied

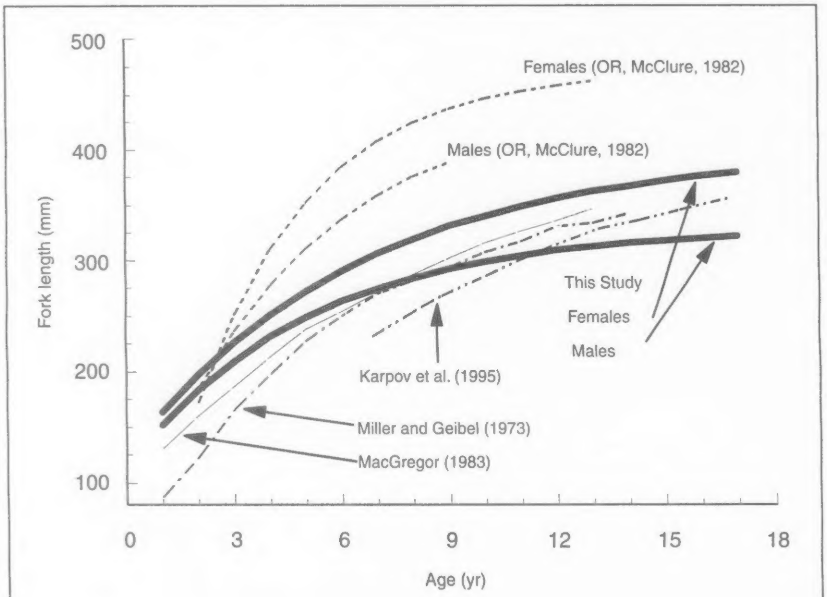


Figure 7

Growth models from five published studies of blue rockfish (*Sebastes mystinus*).

from 0.04/year for female silvergrey rockfish to 0.62/year for the shorter-lived dwarf Puget Sound rockfish, with the average range of *k* values occurring from 0.1 to 0.3/years (Love et al., 1990; Beckman et al., 1998). This considerable longevity and relatively slow growth rate have significant effects on the ability of many rockfish stocks to withstand exploitation.

The age and growth relationships described in this study indicate that both recruitment of blue rockfish to the fishery and their maturity occur at younger ages than previously reported. Blue rockfish enter the fishery at a size of approximately 200 mm (Miller et al., 1967; Miller and Geibel, 1973). This length equates to ages of 2–4 years as determined in our study compared to 3–5 years as estimated by Miller et al. (1967). The new estimates for age at which 50% of individuals are mature (using fish lengths from Miller and Geibel, 1973) are even more striking: our estimated age at 50% maturity is 5–6 years for males and 5 years for females, whereas estimates from Miller and Geibel (1973) and Echeverria (1986) were 7 years for males and 7–8 years for females. Similarly, the youngest mature males and females in these early studies were 4–5 years, whereas we estimated the age to be 3 years.

The changes observed in our study in age-at-length, maximum age, recruitment age, and age at 50% maturity have important implications for stock assessments. Accurate information on age composition, weight-at-age, age specific availability to the fishery, and maturity-at-age is crucial to the proper functioning of the stock synthesis model (Methot, 1990), which is used for Pacific coast groundfish. If incorrect age data are used, it could lead to erroneous estimates of population size, and subsequently to either overfishing or an unnecessary reduction in allowable catch.

Acknowledgments

We would like to thank all the port samplers who collected the CPFV catch data. We also thank the crew and scientific personnel aboard the RV *David Starr Jordan* for collecting samples. We thank James Chess, Edmund Hobson, Dan Howard, and Kelly Silberberg for braving the cold waters of the Pacific Ocean to collect the nearshore specimens. We also thank Churchill Grimes and Mary Yoklavich for their many constructive comments and the reviewers of this manuscript.

Literature cited

Beamish, R. J., and D. A. Fournier.
1981. A method of comparing the precision of a set of age determinations. Can. J. Fish. Aquat. Sci. 38:982–983.
Beamish, R. J., and G. A. MacFarlane.
1987. Current trends in age determination methodology. *In* Age and growth of fish (R. C. Summerfelt and G. E. Hall, eds), p. 15–42. Iowa State Univ. Press, Ames, IA.
Beckmann, A. T., D. R. Gunderson, B. S. Miller, R. M. Buckley, and B. Goetz.
1998. Reproductive biology, growth, and natural mortality of Puget Sound rockfish, *Sebastes emphaeus* (Starks, 1911). Fish. Bull. 96:352–356.

Boehlert, G. W., and R. F. Kappenman.
1980. Variation of growth with latitude in two species of rockfish (*Sebastes pinniger* and *S. diploproa*) from the northeast Pacific Ocean. Mar. Ecol. Prog. Ser. 3:1–10.

Brown, I. W., and W. D. Sumpton.
1998. Age, growth and mortality of redthroat emperor *Lethrinus miniatus* (Pisces: Lethrinidae) from the southern Great Barrier Reef, Queensland, Australia. Bull. Mar. Sci. 62:905–917.

Cailliet, G. M., A. H. Andrews, E. J. Burton, D. L. Watters, D. E. Kline, and L. A. Ferry-Graham.
2002. Age determination and validation studies of marine fishes: do deep-dwellers live longer? Exp. Gerontology 36: 739–764.

Campana, S. E.
2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish. Biol. 59:197–242.

Chilton, D. E., and R. J. Beamish.
1982. Age determination methods for fishes studied by the groundfish program at the Pacific Biological Station, 102 p. Can. Spec. Publ. Fish. Aquat. Sci. 60.

Crabtree, R. E., and L. H. Bullock.
1998. Age, growth, and reproduction of black grouper, *Mycteroperca bonaci*, in Florida waters. Fish. Bull. 96:735–753.

Draper, N., and H. Smith.
1981. Applied regression analysis, 2d ed., 709 p. John Wiley and Sons, New York, NY.

Echeverria, T.
1986. Sexual dimorphism in four species of rockfish genus *Sebastes* (Scorpaenidae). Environ. Biol. Fishes 15: 181–190.

Echeverria, T., and W. H. Lenarz.
1984. Conversions between total, fork, and standard lengths in 35 species of *Sebastes* from California. Fish. Bull. 82: 249–251.

Fraidenburg, M. E.
1980. Yellowtail rockfish, *Sebastes flavidus*, length and age composition off California, Oregon, and Washington in 1977. Mar. Fish. Rev. 42:54–56.

Hare, S. R., and N. J. Mantua.
2000. Empirical evidence for North Pacific regime shifts in 1977 and 1989. Prog. Oceanogr. 47:103–145

Karpov, K. A., D. P. Albin, and W. H. Van Buskirk.
1995. The marine recreational fishery in northern and central California, 192 p. Calif. Dep. Fish Game Fish Bull. 176.

Kimura, D. K., R. R. Mandapat, and S. L. Oxford.
1979. Method, validity, and variability in the age determination of the yellowtail rockfish (*Sebastes flavidus*) using otoliths. J. Fish. Res. Board Can. 36:377–383.

Lea, R. N., R. D. McAllister, and D. A. VenTresca.
1999. Biological aspects of nearshore rockfishes of the genus *Sebastes* from central California, 109 p. Calif. Dep. Fish Game Fish Bull. 177.

Love, M. S., and K. Johnson.
1998. Aspects of the life histories of grass rockfish, *Sebastes rastrelliger*, and brown rockfish, *S. auriculatus*, from southern California. Fish. Bull. 87:100–109.

Love, M. S., P. Morris, M. McCrae, and R. Collins.
1990. Life history aspects of 19 rockfish species (Scorpaenidae: *Sebastes*) from the Southern California Bight, 38 p. NOAA Tech. Rep. NMFS 87

Love, M. S., M. Yoklavich, and L. Thorsteinson.
2002. The rockfishes of the northeast Pacific, 405 p. Univ. California Press, Berkeley, CA.

MacGregor, J. S.
1983. Growth of the blue rockfish (*Sebastes mystinus*). CALCOFI (Calif. Coop. Ocean. Fish. Investig.) Rep. XXIV: 216–225.

Mayo, R. K., V. M. Gifford, and A. Jearld Jr.
1981. Age validation of redfish, *Sebastes marinus* (L.), from the Gulf of Maine–Georges Bank region. J. Northwest Atl. Fish. Sci. 2:13–19.

McClure, R. E.
1982. Neritic reef fishes off central Oregon: aspects of life histories and recreational fishery. M.S. thesis, 94 p. Oregon State Univ., Corvallis, OR.

Methot, R. D.
1990. Synthesis model: an adaptable framework for analysis of diverse stock assessment data. Bull. Int. North Pac. Fish. Comm. 50:259–277.

Miller, D. J., and J. J. Geibel.
1973. Summary of blue rockfish and lingcod life histories; a reef ecology study; and giant kelp, *Macrocystis pyrifera*, experiments in Monterey Bay, California, 137 p. Calif. Dep. Fish Game Fish Bull. 158.

Miller, D. J., M. W. Odemar, and D. W. Gotshall.
1967. Life history and catch analysis of the blue rockfish (*Sebastodes mystinus*) off central California, 1961–1965. Calif. Dep. Fish Game Mar. Res. Operations Ref. 67-14: 1–130.

Munk, K. M.
2001. Maximum ages of groundfish in waters off Alaska and British Columbia and considerations of age determination. Alaska Fish. Res. Bull. 8:12–21.

Pearson, D. E.
1996. Timing of hyaline-zone formation as related to sex, location, and year of capture in otoliths of the widow rockfish, *Sebastes entomelas*. Fish. Bull. 94:190–197.

Pearson, D. E., and J. E. Hightower.
1991. Spatial and temporal variability in growth of widow rockfish (*Sebastes entomelas*), 47 p. NOAA Tech. Memo., NOAA-TM-NMFS-SWFSC 167.

Pearson, D. E., J. E. Hightower, and J. T. H. Chan.
1991. Age, growth, and potential yield for shortbelly rockfish, *Sebastes jordani*. Fish. Bull. 89:403–409.

PFMC (Pacific Fishery Management Council).
2001. Status of the Pacific coast groundfish fishery through 2001 and recommended acceptable biological catches for 2002. Pacific Fishery Management Council, Portland, OR.

Ratkowsky, D. A.
1983. Nonlinear regression modeling, 276 p. Marcel Dekker, New York, NY.

Rogers, J. B., M. Wilkins, D. Kamikawa, F. Wallace, T. Builder, M. Zimmerman, M. Kander, and B. Culver.
1996. Status of the remaining rockfish in the *Sebastes* complex in 1996 and recommendations for management in 1997. Appendix E: Status of the Pacific coast groundfish fishery through 1996 and recommended biological catches for 1997: stock assessment and fishery evaluation, 59 p. Pacific Fishery Management Council, Portland, OR.

Schnute, J.
1981. A versatile growth model with statistically stable parameters. Can. J. Fish. Aquat. Sci. 38:1128–1140.

Six, L. D., and H. F. Horton.
1977. Analysis of age determination methods for yellowtail rockfish, canary rockfish, and black rockfish off Oregon. Fish. Bull. 75:405–414.

Stanley, R. D., and B. R. Melteff.
1987. A comparison of age estimates derived from the surface and cross-section method of otolith reading for Pacific ocean perch (*Sebastes alutus*). Lowell Wakefield fisheries symposium: proceedings of the international rockfish symposium, Anchorage, Alaska, USA, Oct. 20–22, 1986 Alaska Sea Grant Rep. 87-2, p. 187–196. Univ. Alaska, Anchorage. AK.

Wales, J. H.
1952. Life history of the blue rockfish, *Sebastodes mystinus*. Calif. Fish Game 38(4):485–498.

Wilson, C. D., and G. W. Boehlert.
1990. The effects of different otolith ageing techniques on estimates of growth and mortality for the splitnose rockfish, *Sebastes diploproa*, and canary rockfish, *S. Pinniger*. Calif. Fish Game 76:146–160.

Reproduction and recruitment of white mullet (*Mugil curema*) to a tropical lagoon (Margarita Island, Venezuela) as revealed by otolith microstructure*

Baumar J. Marin E.
Antonio Quintero
Instituto Oceanográfico de Venezuela
Universidad de Oriente
Cumaná 6101
Edado Sucre, Venezuela
E-mail address (for B. J. Marin E.): bmarin@sucre.udo.edu.ve

Dany Bussière
Julian J. Dodson
Département de biologie
Université Laval,
Ste-Foy
Québec, Canada G1K 7P4

Abstract—The reproductive activity and recruitment of white mullet (*Mugil curema*) was determined by observations of gonad development and coastal juvenile abundance from March 1992 to July 1993. Adults were collected from commercial catches at three sites in northeastern Venezuelan waters. Spawning time was determined from the observation of macroscopic gonadal stages. Coastal recruitment was determined from fish samples collected biweekly by seining in La Restinga Lagoon, Margarita Island, Venezuela. The examination of daily growth rings on the otoliths of coastal recruits was used to determine their birth date and estimate the period of successful spawning. Fish with mature gonads were present throughout the year but were less frequent between September and January when spawning individuals migrated offshore. In both years, juvenile recruitment to the lagoon was highest between March and June when high densities of 25–35 mm juveniles were observed. Back-calculated hatching-date frequency distributions revealed maximum levels of successful spawning in December–January that were significantly correlated with periods of enhanced upwelling. The relation between the timing of successful spawning and the intensity of coastal recruitment in white mullet was likely due to variations in food availability for first-feeding larvae as well as to variations in the duration of the transport of larvae shoreward as a result of varying current conditions associated with upwelling.

Manuscript approved for publication 10 June 2003 by Scientific Editor.
Manuscript received 26 June 2003 at NMFS Scientific Publications Office.
Fish. Bull. 101:809–821

White mullet (*Mugil curema*) is a widespread coastal pelagic fish occurring from Massachussetts to southern Brazil. Considered to be catadromous, the juvenile fish recruit to lagoons and estuaries following a period of offshore spawning (Blaber, 1987; Ibañez-Aguirre, 1993; Ditty and Shaw, 1996). White mullet is an important economic resource supporting many small communities through both fishing and aquaculture (Alvarez-Lajonchere, 1982; Gómez and Cervigón, 1987). Small schools of mullet are captured with gill and "atarraya" nets near the coast and in neritic waters and between 300 and 400 metric tons are sold annually on Margarita Island, Venezuela.

Reproductive periodicity in white mullet varies over its geographic distribution. Several authors have reported protracted or continuous reproduction in tropical waters and generally two spawning peaks per year (Jacot, 1920; Anderson, 1957; Angell, 1973; Moore, 1974; Alvarez-Lajonchere, 1976, 1980; Yañez-Arancibia, 1976; Rodriguez and Nascimento, 1980; Garcia and Bustamente, 1981; Franco, 1986; Ibanez-Aguirre, 1993). Figure 1 summarizes previous work describing the spawning periods of *M. curema* based on gonad development and estimated according to the arrival of juveniles in the coastal zones. The spawning period is quite variable. Angell (1973) suggested that schooling occurs in coastal areas just prior to the offshore spawning migration and that the departure of individuals for the spawning grounds causes a reduction of the gonadosomatic index in the nearshore populations. Moore (1974) also reported that during the spawning period fully ripe fish are rare in coastal collections. Despite these studies, little is known of the factors influencing reproductive patterns of the white mullet. Ibanez-Aguirre (1993) suggested that the timing of reproduction in *M. curema* in Tamiahua Lagoon, Mexico, is an adaptation to avoid competition with juveniles of the conspecific *Mugil cephalus*. In areas of favorable thermal regimes, *M. curema* may penetrate a wider range of salinities and competitively exclude *M. cephalus* (Moore, 1974).

The periodicity of white mullet reproduction may be related to environmental variability that signals periods of optimal early growth and survival. Stability of the water column and suit-

---

* Contribution of Québec-Océan, Pavillon Alexandre-Vachon, Local 2078, Université Laval, Québec, Qc. GIK 7P4.

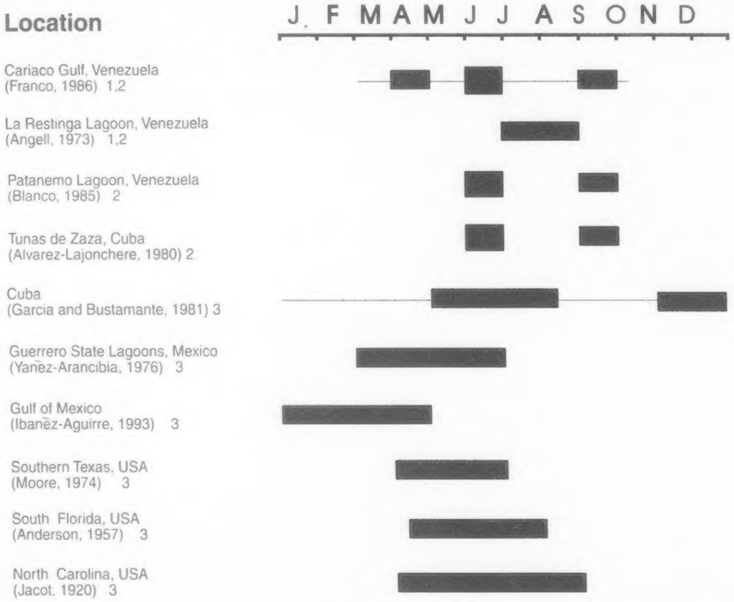


Figure 1

Spawning periods for white mullet (*Mugil curema*) in tropical waters, based on the histological observations of gonads (1), macroscopic observations of gonads (2), and estimated from the arrival of juveniles in coastal areas (3). Thin horizontal lines indicate periods of continuous but minor spawning between periods of peak spawning.

able food in coastal lagoons, river deltas, and estuarine mangrove areas have been identified as important factors influencing the recruitment of juvenile Mugilidae (Yañez-Arancibia, 1976; Blaber and Blaber, 1980; Blaber, 1987; Vieira, 1991). Based on macroscopic gonad observations of schools of white mullet captured offshore, Etchevers (1974) proposed that the spawning of white mullet recruiting along the southern coast of Margarita Island, Venezuela, occurs between La Tortuga Island and Margarita Island in the vicinity of the 1000-m deep Cariaco trench (Fig. 2). Seasonal environmental variability in this area is mainly generated by the alternation between upwelling during the dry season and freshwater discharge during the wet season (Gómez, 1983; Müller-Karger et al., 1989). The rainy season strongly influences the eastern Caribbean as freshwater plumes from the Amazon and Orinoco Rivers lower salinities throughout the region. Both upwelling and freshwater runoff produce intense peaks in coastal primary production (Gines 1972; Ferraz-Reyes et al., 1987; Müller-Karger et al., 1989), which could influence spawning periodicity and recruitment success. The purpose of this study was to document the periodicity of reproduction and recruitment of *M. curema* along the southern coast of Margarita Island and to examine their relationship with environmental signals, particularly those associated with upwelling.

Methodological advances in counting daily growth increments in otoliths of marine fishes (Pannella, 1971; Campana and Nielsen, 1985) have greatly aided studies of the age, growth, and recruitment of larval and juvenile fishes (Wilson and Larkin, 1980; McBride and Conover, 1991; Jenkins and May, 1994; Sirois and Dodson, 2000). For the striped mullet (*M. cephalus*), a close relative of the white mullet, Radtke (1984) showed that the first increment is formed one day after hatching and that additional increments are formed daily thereafter. Daily growth rings have also been demonstrated in laboratory studies for *M. so-iuy* by Li et al. (1993). In the present study, we examined the microstructure of the otoliths of juveniles recruiting to a coastal lagoon in order to back-calculate the date of hatching and hence the time of successful spawning. We first validated that otolith growth increments of juvenile *M. curema* were formed daily.

Material and methods

Reproductive periodicity was documented from samples of adult fish taken monthly from commercial catches in three fishery zones in Venezuela: 1) the Chacopata zone, located between Chacopata lagoon and Coche and Cubagua Islands; 2) the Cariaco Gulf zone; and 3) the Margarita zone located along the southern coast of Margarita Island and the northern coast of Cubagua Island (Fig. 2). Measurements of water temperature, salinity, and rainfall were

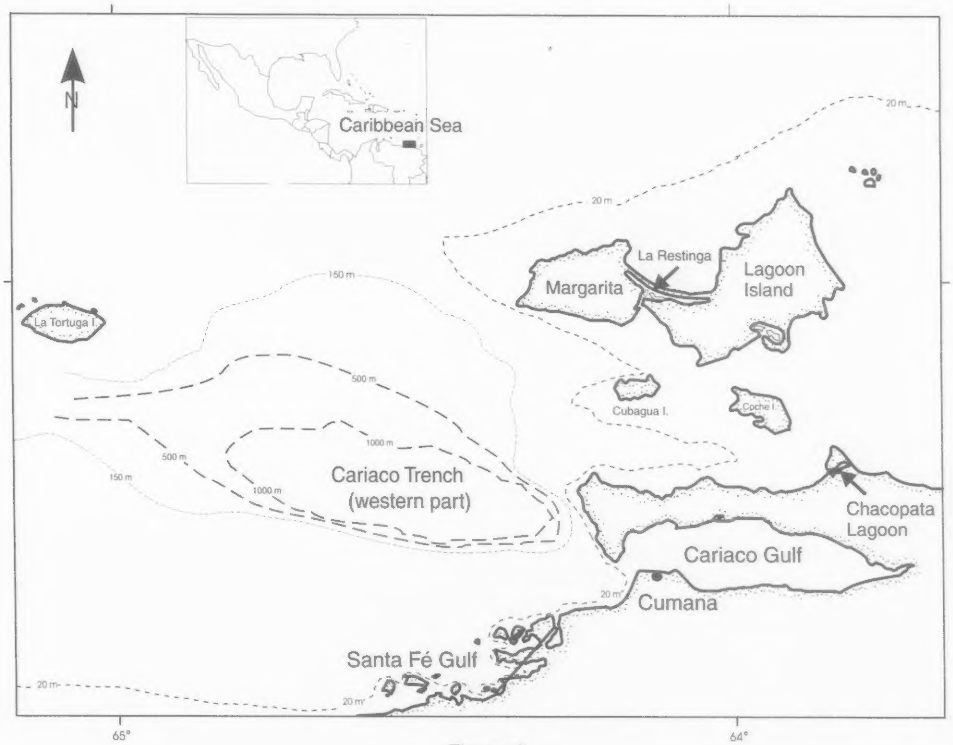


Figure 2

Map of the northeastern coast of Venezuela showing locations mentioned in the text.

collected periodically throughout the entire study period at the La Salle meteorological station, next to La Restinga Lagoon on Margarita Island.

Total (TL) and standard (SL) lengths of adult mullet were measured to the nearest 0.5 cm and total and gutted body mass were recorded to the nearest 0.1 g. Sexual maturity was determined by observation of the gonads and gonadal stages were classified as follows:

Stage I	Ovaries transparent and inconspicuous, whitish-yellow in color and rounded with a small diameter. Testes longer than ovaries and ribbonlike in form.
Stage II	Ovaries rounder and wider than in stage I, and yellow in color. Testes thinner, and wider than stage I, but still with thin edges and a ribbonlike form; white in color.
Stage III	Ovaries large, pale yellow, smooth in appearance, turgid, and round. Ovocytes easily distinguished macroscopically (as granular). Testes milky-white in color (bright), turgid, and wider in appearance and having thicker edges than in stage II.
Stage IV	Spawned (spent) ovaries purple and wrinkled in appearance. Testes whitish, or transparent with white patches, and wrinkled in appearance.

Recruitment periodicity was documented from samples of juveniles seined at semimonthly intervals at the mouth of La Restinga Lagoon (Fig. 2). The 2-cm mesh beach seine measured 1.5 m deep and 50 m long. Juvenile white mullet were distinguished from other juvenile mullets according to the descriptions of Alvarez-Lajonchere et al. (1976). White mullet juveniles were characterized by a scaly gray appearance as opposed to the shiny metallic gray appearance of a sympatric mullet species (*Mugil incilis*). For white mullet, recruitment is defined as the appearance of juveniles in coastal areas (Vieira, 1991). We calculated catch per unit of effort (CPUE) as the number of juveniles per seine haul. For all samples, standard length of fish was measured to the nearest 1 mm. Otolith analysis was restricted to one sampling period per month. After examining size-frequency distributions of juveniles captured in the lagoon, the otoliths of approximately 20 individuals representing all cohorts collected on a given sampling date were analyzed. The otoliths (sagittae) were removed with needles, rinsed in water, and then attached to strips of masking tape. The otolith was then sanded to obtain a transversal section (Fig. 3) with a thickness of approximately 20 μm by using the technique described by Secor et al. (1992) and a metallurgic jig adapted from Neilson and Geen (1986). Readings of the number of increments were made along the curvilinear surface running from the nucleus to the edge of the otolith (Fig. 3). Because daily growth increments were

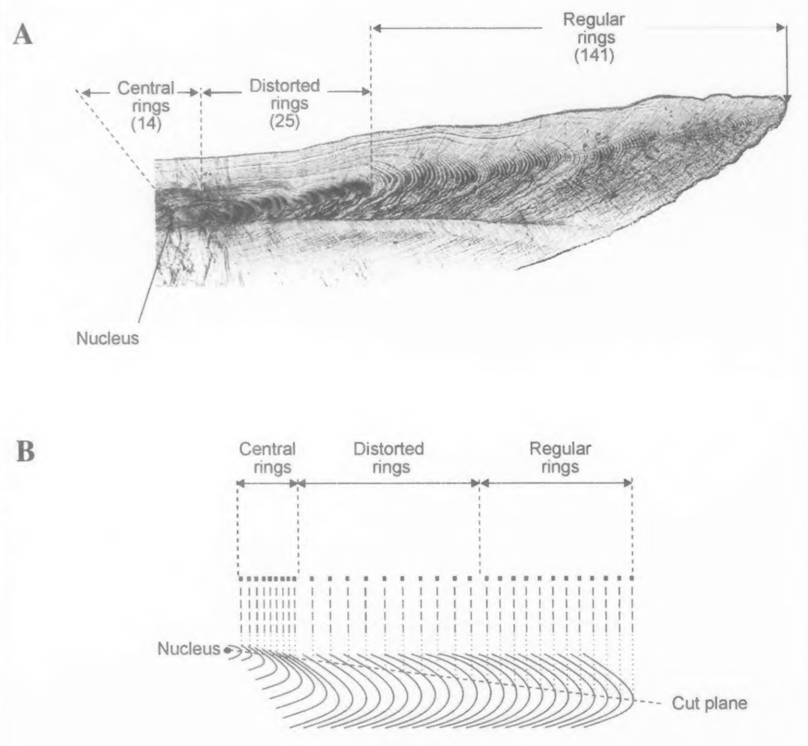


Figure 3

(**A**) Photomicrograph in optical microscopy of the posterior radius of a polished otolith from a 180-day-old juvenile white mullet (*Mugil curema*). Different sequences of rings are indicated which result from a horizontal plane not aligned with the changing growth plane of the otolith. Numbers of rings in each sequence are shown in parentheses. (**B**) Schematic presentation of the changing growth plane of the otolith, the cut plane, and the resultant increment distortion (black rectangles represent the distance between adjacent rings).

less consistent in the anterior field, otolith counts were always made along the posterior radius of the sagittae. We also measured the area of the nucleus, which represents the prehatch zone. All measurements were made under a microscope which was connected to an image analyzer and computer.

To evaluate the error in counting growth increments on otoliths, one reader made 7 independent counts of the number of growth increments on otoliths obtained from 11 juveniles representing the size-range of sampled fish. The number of growth increments ranged from 63 to 289 (mean=147) and the mean coefficient of variation was 8.71% (SD=2.13%) (Fig.4). We therefore considered an error of approximately 10% for the counts of growth increments. In applying the technique to the subsamples of the different cohorts sampled in the lagoon, at least two counts were made for each otolith. All counts were made by the same person.

To evaluate if otolith growth increments were formed daily, we read the otoliths of juveniles sampled on successive sampling dates and compared the average increase in the number of otolith increments to the number of days between samplings (Struhsaker and Uchiyama, 1976; Jordan, 1993; Jenkins and May, 1994). Birthdate was obtained by subtracting the number of daily growth rings on otoliths from the date of capture. We used the hatching mark as defined for *M. cephalus* (Radtke, 1984) and *M. iso-iuy* (Li et al., 1993) to locate the hatching mark on the otoliths of the white mullet.

Knowing that white mullet embryos hatch from 24 to 40 hours after fertilization (Anderson, 1957; Houde et al., 1976), we back-calculated hatching dates of recruits to estimate when successful spawning occurred. We examined the relationship between the spawning dates of recruits and an index of the intensity of upwelling. In calculating this index, we determined wind stress based on data from Fundación La Salle, Margarita Island, and Cumaná Airport meteorological stations. The upwelling index (UI) was based on Bowden's (1983) theoretical calculations as follows:

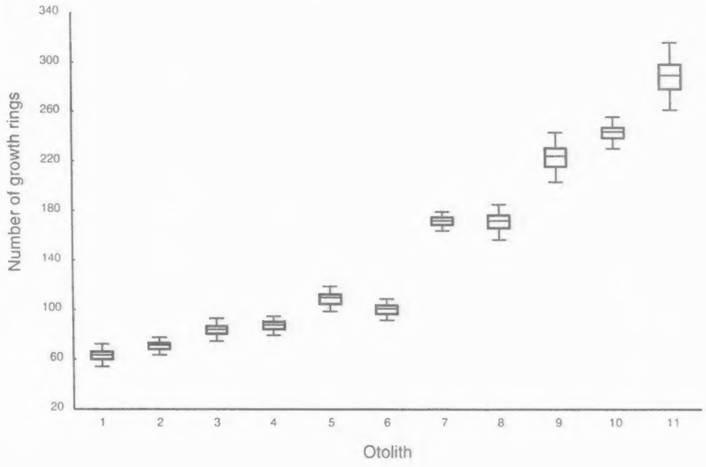


Figure 4

Number of growth increments (counted on seven independent occasions) on otoliths from 11 juvenile white mullet of varying size. Means, standard errors, and standard deviations are illustrated.

$$\mathrm{UI} = \frac{\tau_{sx}.100}{\rho_w.f},$$

where f = Coriolis parameter;
τ_{sx} = surface wind stress; and
ρ_w = average density of the water (1025 kg/m^3).

The term f was calculated as

$$f = 2\ \omega \sin(f_i),$$

where ω = angular velocity of rotation of the earth (7.29×10^{-5} s [seconds]): and
f_i = latitudinal position at the place i.

The term τ_{sx} represents surface wind stress measured in the x-axis perpendicular to the coast (Bowden, 1983), often considered in terms of the empirical equation

$$\tau_{sx} = k \times \rho_a \times W^2,$$

where k = empirical drag coefficient (1.11 to 3.25, as a function of wind velocity; Bowden 1983);
ρ_a = mean density of the air (1.25 kg/m^3); and
W = wind velocity.

The drag coefficient, k, changes as a function of wind velocity and gives values equivalent to those of Bakun et al. (1974).

The relationship between upwelling and the birth dates of successful recruits (captured in La Restinga Lagoon) was determined in two steps. First, we calculated the birth dates of juveniles captured during one monthly sampling period during each of the 18 months of the study by applying the frequency distribution of birth dates of aged juveniles to the total catch for that date. A total of 398 juveniles were aged by otolith analysis. If x% of aged fish captured on a given date were hatched on Julian Day y, this percentage was applied to the total catch of juveniles for that sampling date. Secondly, all fish hatched on a given day were summed across the 18 monthly sampling dates. This frequency distribution was then correlated with the distribution of UI estimates over the same period of time as that of the birth dates.

Before proceeding with correlation, trends in birth date and UI data series were described by using a smoothing spline. The spline fit uses a set of smoothly spliced 3rd degree polynomial segments (Simonoff, 1996; JMP® software, version 3.2.1, SAS Institute, Cary, North Carolina). Predicted values were correlated with the raw data points in order to optimize the value of lambda used to fit the smoothing spline. Increasing the value of lambda increases the degree of smoothing but weakens the correlation between predicted and raw data. Pearson correlation and cross-correlation functions were used to describe the temporal relationship between upwelling and the date of hatching of fish recruited to the coastal lagoon.

Results

The white mullet surveyed in the commercial catches measured from 4 to 36 cm SL. The largest fish were from the Chacopata zone where the most abundant sizes classes were those from 22 to 30 cm. The most abundant sizes in the Cariaco Gulf and Margarita Island zones were 20 to

26 cm and 18 to 26 cm, respectively. The mullet from the Margarita zone were mainly juveniles and small adults (Fig. 5).

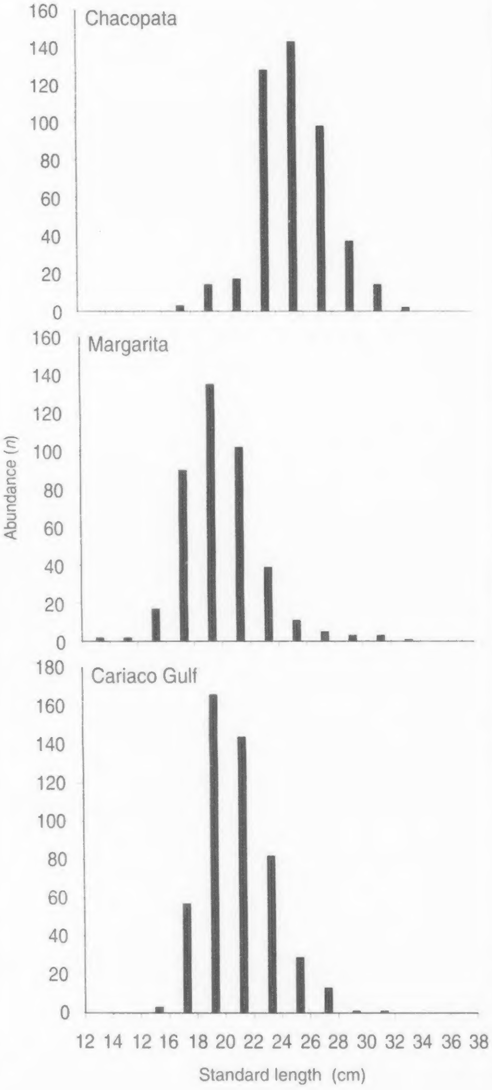


Figure 5

Length-frequency distributions of white mullet surveyed in the commercial catches from the three fishery zones of northeast Venezuela.

Maturity and reproductive periodicity

An examination of gonad maturity revealed that 90% of the male and female mullet in the Margarita zone were immature or at developing stages (I and II) and only 10% in developed and spawning stages (III and IV). In contrast, 53.8% of females and 42.8% of males of the population of generally larger mullet sampled at Chacopata were in developed and spawning stages. Finally in the Cariaco Gulf zone, where the fish were of intermediate size, compared to fish at Margarita and Chacopata, 41.2% of females and 32.7% of males were in developed and spawning stages.

Throughout our study, sexually mature (stage-III) fish were present in the samples from the Chacopata fishery (Fig. 6), and their abundance showed a marked seasonal pattern. Mature and spent fish were least abundant (<25% of the population) between September and January and most abundant from April to August 1992 and May to June 1993. In contrast, in the Margarita fishery, immature (stage-I) fish dominated the samples and mature fish only occurred sporadically (Fig. 6). Finally, in the Cariaco Gulf zone, immature and maturing fish (stage-I, and stage-II) generally dominated the population, except in July when mature fish became abundant.

Otolith microstructure

The otolith of *M. curema* had a round nucleus with a mean radius of 9.26 μm (95% confidence interval (CI)=0.54, n=8), and the dark area in the center had a mean diameter of 4.97 μm (CI=0.82, n=8). The otolith was round during the larval period and became ovoid when mullet reached 10–12 mm (SL). In the early juvenile stage (18–20 mm SL), the otolith was strongly elongated and the anterior end was arrow-like. In juveniles (>20 mm SL), the otolith was always thin, concave, and umbrella shaped in form (Fig. 3).

Validation of otolith increment lines

The otolith increments counted for the first strong cohort present in the lagoon during March and April 1992 demonstrated that the average number of increments added during the 14-day interval between sampling collections was close to 14 days (Table 1). This indicated that otolith increments were formed daily, as observed in other species of mullet.

Juvenile recruitment

The catch-per-unit-of-effort measurements for juveniles captured in La Restinga Lagoon demonstrated a seasonal pattern and high recruitment from March to early July 1992 and from late March to May 1993, and low recruitment during the remaining months (Fig. 7). The recruitment peak in 1992 was more than twice that in 1993. The periods of strong recruitment were associated with the rainy season in northeastern Venezuela (Fig. 7), as previously reported by Okuda et al. (1978), Gómez (1983), and Ferraz-Reyes (1989).

The discontinuous length-frequency distributions of juveniles sampled biweekly suggested the presence of four cohorts in the lagoon during the study period (Fig. 8). Two cohorts were present on 5 March 1992, the first sampling date. The cohort of smaller juveniles, referred to as the first cohort, had a mean length of 29.8 mm (range of 18 to

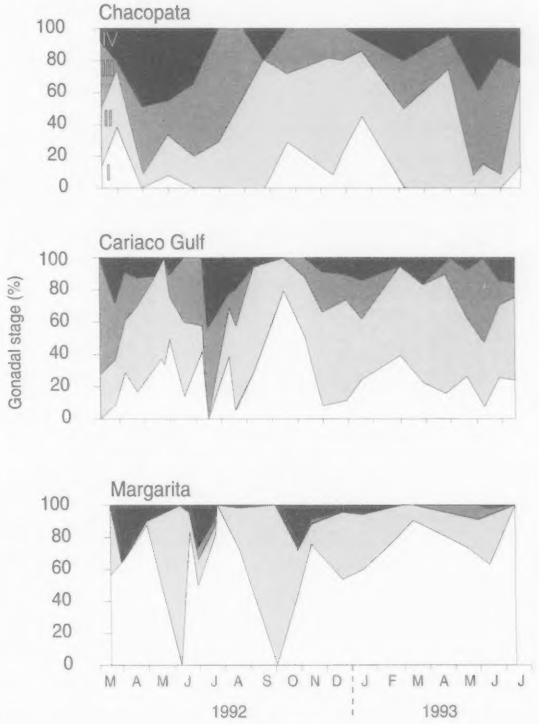


Figure 6

Percent composition of four maturity stages (I to IV, see text for definitions) of *Mugil curema* (males and females combined) captured from Margarita, the Cariaco Gulf, and the Chacopata fishery zones of northeast Venezuela.

36 mm) and varied in age from 50 to 70 days (Fig. 9). The cohort of larger juveniles, referred to as the second cohort, had a mean length of 105 mm (range of 90 to 130 mm), and otolith analysis indicated an age of 160 to 240 days (Fig. 8). This cohort was present until May 1992 and was largely absent thereafter (Fig. 7). A third cohort was first observed in mid October 1992. It measured from 50 to 100 mm in length, overlapping the length distribution of cohort 1. As such, no clear distinction could be made between these two cohorts on these dates. The third cohort became more distinct in December 1992 and January 1993 as individuals from the first cohort left the lagoon. These individuals were aged from 95 to 200 days old in December. This cohort was present until approximately April 1993 and disappeared thereafter (Fig. 9). Finally, a fourth cohort first appeared in March 1993 and consisted of fish of similar size and age as the first cohort observed in March of the previous year.

The back-calculated hatching dates showed that the second, older cohort observed in the lagoon in March 1992 was composed of mullet that had hatched between August and October 1991 (Fig. 10). The younger cohort in the March 1992 sample originated from continuous hatching from late December 1991 to late March 1992. The back-calculated hatching dates indicated that larval production of successful recruits was almost absent during April and May 1992 but small increases were observed in June and July 1992. The third cohort, which first appeared in September 1992, was

Table 1

Validation of daily increment formation in the otoliths of juvenile white mullet (*Mugil curema*) sampled at 14-day intervals in March and April 1992. n= number of otoliths in sample, mean age (standard deviation) is given in days on date of capture, and "difference" is the difference in mean age between successive sampling dates.

Sampling dates	5 March 1992	19 March 1992	2 April 2 1992
n	15	20	19
Mean age (SD)	56.46 (4.81)	70.85 (9.88)	84.3 (11.24)
Difference		14.39	13.45

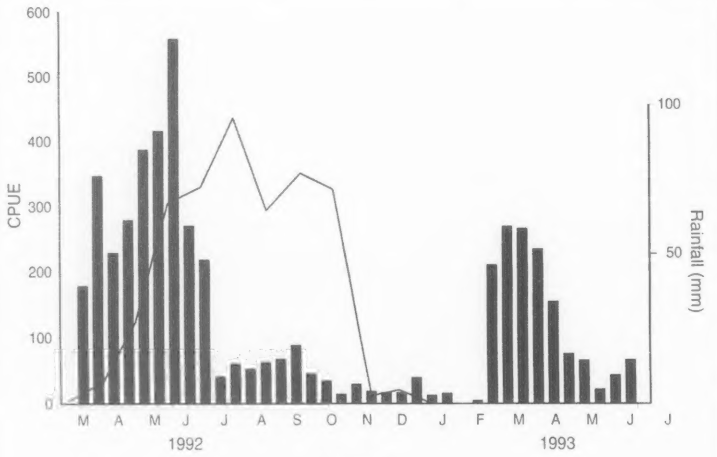


Figure 7

Histogram of monthly abundance of juvenile white mullet as indicated by catch per unit of effort (CPUE) from semimonthly sampling in La Restinga lagoon from March 1992 to July 1993. The continuous black line presented in the first annual cycle represents the mean monthly rainfall averaged over the period 1985–93.

Table 2

Correlation analyses of hatching date and upwelling index data series. Increasing lambda values used to fit the smoothing spline weakens the r^2 values between observed and predicted values of birth date and upwelling index over time (columns 2 and 3). In contrast, increasing lambda values strengthen the correlation (Pearson's correlation) between the two data series (column 4). BD = birth date, UI = upwelling index

Data series	Hatching dates (r^2, P)	Upwelling index (r^2, P)	BD versus UI (r, P)
Raw data	—	—	0.28, <0.05
Smoothing spline $\lambda = 1$	0.82, <0.000	0.86, <0.000	0.36, <0.000
Smoothing spline $\lambda = 10$	0.73, <0.000	0.74, <0.000	0.41, <0.000
Smoothing spline $\lambda = 100$	0.67, <0.000	0.63, <0.000	0.45, <0.000
Smoothing spline $\lambda = 1000$	0.64, <0.000	0.54, <0.000	0.52, <0.000
Smoothing spline $\lambda = 10{,}000$	0.56, <0.000	0.45, <0.000	0.57, <0.000
Smoothing spline $\lambda = 100{,}000$	0.48, <0.000	0.40, <0.000	0.64, <0.000

composed of individuals that had hatched between June and August 1992. Finally, individuals in the fourth cohort, which first appeared in the lagoon in March 1993, were fish that had hatched in January and February 1993.

The hatching dates of recruits coincided with periods of increasing upwelling, particularly during January and February of 1992 and 1993 (Fig. 10). The use of increasing levels of lambda to fit the smoothing spline increasingly weakened the correlations between predicted and observed values of birth dates and UI index and strengthened the correlations between birth dates and the upwelling index (Table 2). Choosing a lambda value of 10 resulted in r^2 values greater than 0.70 ($P<0.000$) between the raw and predicted data series and in an r value of 0.41 ($P<0.000$) between birth date and the upwelling index. Cross-correlation analysis between these two series revealed that the strongest correlation ($r=0.52$, $P<0.000$) occurred when the upwelling index lagged behind birth dates by 8 days and by 46 days. These lag periods reflect the coincidence of the major peak of upwelling with the two peaks of birth dates that are separated by approximately 35 days. Given the estimated 10-day error associated with aging otoliths, the 8-day lag cannot be interpreted.

Discussion

Our sampling of white mullet in the coastal waters of northeastern Venezuela revealed the presence of mature fish throughout the year, but abundance was lowest between August and January. Mullet from the Margarita zone were small (4 to 36 cm in SL) and mostly immature (>80%). Because size at maturity of white mullet is 24 cm (Marin and Dodson, unpubl. data), most of the adults in the lagoon were probably in their first spawning cycle. Similarly, mullet from the Cariaco Gulf zone also appeared to be young adults in their first spawning cycle. In contrast, mullet from the Chacopata zone were larger and generally in more advanced stages of gonadal development. This finding suggests that the Chacopata mullet were part of a prespawning aggregation, and the location of the aggregation agrees with the more offshore location of the Chacopata fishery.

Because white mullet spawn offshore (Jacot, 1920; Anderson, 1957; Ditty and Shaw, 1996), the small proportion of mature fish in the coastal fisheries from July to April is likely explained by the migration of adults to the offshore spawning grounds. If this is so, reproduction is not indicated by an increase in the frequency of fish in advanced stages, but rather is associated with the disappearance of maturing and mature fish from coastal areas. The disappearance of fish in advanced stages from coastal areas as the spawning season approaches was also reported by Angell (1973) and Moore (1974). The analysis of birth dates of juveniles sampled in the La Restinga Lagoon indicates that successful spawnings are concentrated in the periods of increased upwelling and also coincide with the end of the rainy season. The spawning season may or may not be concentrated at these times but larvae that hatch during upwelling events are most likely to successfully recruit to the lagoon.

Although reproduction in tropical fishes is often protracted, peaks in successful spawning may nevertheless be initiated by environmental cues (Redding and Patiño, 1993). The white mullet possibly uses temperature or other signals associated with upwelling to synchronize its spawning with upwelling events. The variations in the timing of recruitment of white mullet in different geographical regions may be the result of variation in the timing of favorable conditions that enhance survival. Such conditions may include increased primary production (Ferraz-Reyes, 1983; Müller-Karger et al., 1989) or hydrographic mechanisms likely to facilitate transport of larvae to coastal nursery areas (Blaber, 1987), so that survival is increased. Populations likely have adequate time to adapt to environmental conditions in particular areas because local hydrographic patterns develop over geological time scales (Bakun, 1986; Sinclair, 1988; Heath, 1992).

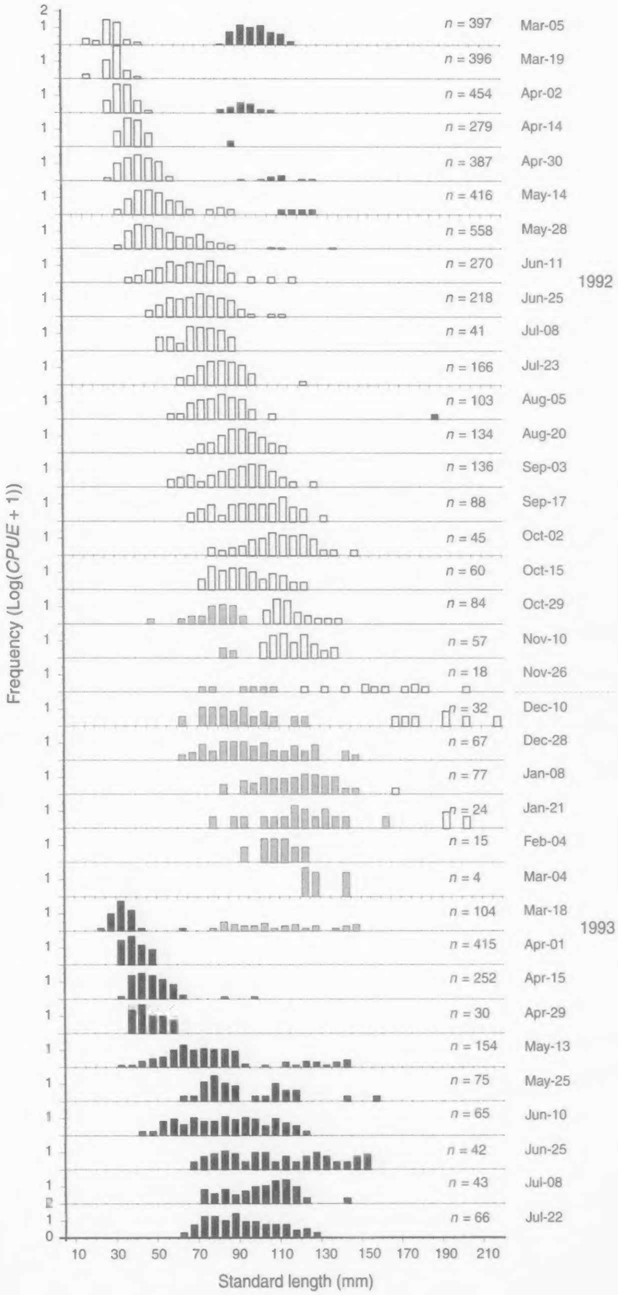


Figure 8

Semimonthly size distributions of juvenile white mullet from March 1992 to July 1993 in La Restinga Lagoon. The abundance for each size class is presented as the Log CPUE + 1. From top to bottom, open bars represent cohort 1, black bars represent cohort 2, gray bars represent cohort 3, and black bars represent cohort 4. Cohorts were identified by discontinuities in size distributions of juveniles. *n* = number of fish sampled.

Strong offshore transport of surface waters occurs during upwelling events, so that a rapid metamorphosis to the demersal stage may be critical for the coastal recruitment of white mullet. This rapid metamorphosis is suggested for several offshore spawning fishes with pelagic larvae that subsequently recruit to estuaries (Creutzberg et al., 1978; Heath, 1992) or that remain near the bottom during ebb flow, once close to the coast, thereby reducing offshore transport (Bartsch and Knust, 1994). White mullet undergo metamorphosis to the demersal stage 14 days after hatching (Houde et al., 1976) at which time they would be entrained in the inshore transported water that occurs at depths greater than 50 m in the coastal zone of northeastern Venezuela (Quintero, unpubl. data). Several studies suggest that increased mortality is caused by increased predation associated with the change to bottom habitat (Johannes, 1978; Bakun, 1986). Heath (1992) suggested that mortality from predation is particularly high during migration to nursery areas. Given the time to metamorphosis (14 days) and the age of white mullet when they enter the lagoon (50 to 70 days for the first cohort), metamorphosis to the demersal stage most probably occurs at least one month before entry into the lagoon (Anderson, 1957; Caldwell and Anderson, 1959; Yañez-Arancibia, 1976; Vieira, 1991).

During the demersal period at sea, white mullet may be exposed to considerable mortality due to benthic predators. Variation in the abundance of recruitment pulses into La Restinga Lagoon may reflect the interplay between spawning time and the mortality during transport to the coastal area. At some point between metamorphosis and lagoon entry, juvenile mullet also develop active swimming behavior to facilitate passive transport. We observed intensive recruitment of small mullet into the lagoon between March and June by individuals that had hatched the previous December to February. The timing of their hatching means that their return to the lagoon was likely facilitated by prevailing currents. In contrast, recruitment of mullet to the lagoon over the remainder of the year was weak and sporadic, and fish were much larger and older. At its first appearance in the lagoon, the third cohort was twice the age of the first cohort. These fish were not produced during a period when currents would likely have facilitated larval transport to the lagoon (little upwelling) and their lower densities may partially reflect increased mortality during the more prolonged return to the lagoon. We propose that spawning during periods of weak upwelling causes a delay in transport to coastal nursery areas and consequently decreased survival.

Periods of hatching leading to successful recruitment, from late December to March, coincided with moderate peaks in the upwelling index. This successful recruitment may be the result of moderate levels of wind speed (<6 m/s^2), that promote moderate upwelling and yield optimal trophic conditions for fish larvae (Cury and Roy, 1989). Coastal upwellings in northeast Venezuelan waters are caused by

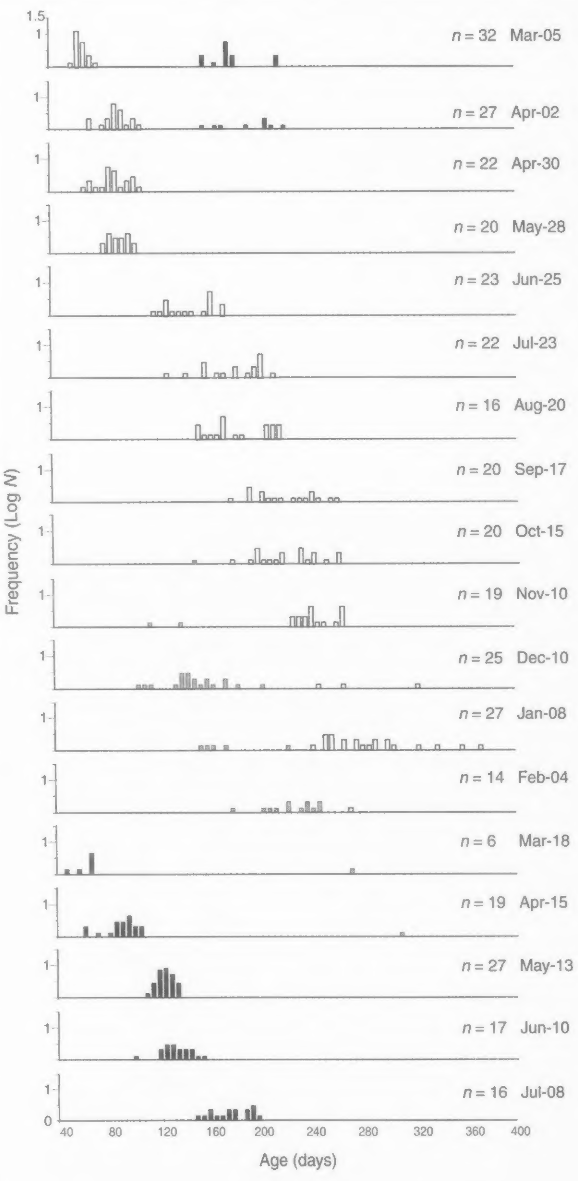


Figure 9

Monthly age distributions of juvenile white mullet from March 1992 to July 1993. Age was calculated by counting daily growth rings starting with the hatch mark. From top to bottom, open bars represent cohort 1, black bars represent cohort 2, gray bars represent cohort 3 and black bars represent cohort 4. *n* = number of otoliths examined.

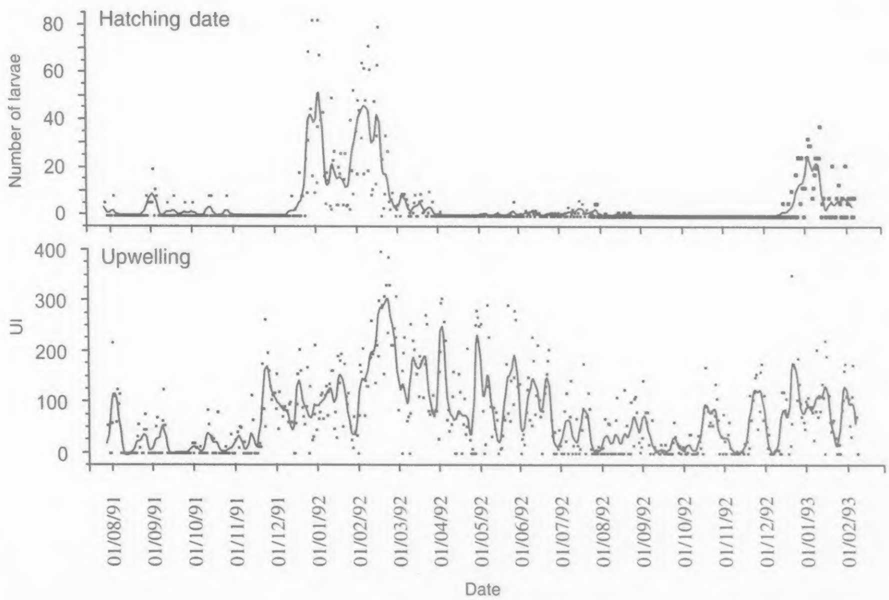


Figure 10

(**Upper panel**) Frequency distribution of hatching dates for juvenile white mullet recruits caught in 1992 and 1993 in La Restinga lagoon. Points represent raw data. Continuous black line represents the trend line generated after application of a smoothing spline (lambda=10). (**Lower panel**) Upwelling index (UI) for northeastern Venezuela calculated from August 1991 to March 1993. See text for description of calculation. Points represent raw data. Continuous black line represents the trend line generated following application of a smoothing spline (lambda=10).

moderate levels of wind stress and this differs from the strong upwellings observed in such places as Peru and Senegal. The relation between the timing of successful spawning and the intensity of coastal recruitment in white mullet is likely due to variations in the duration of the transport of larvae and juveniles to the shore as a result of varying current conditions as well as variations in food availability for first-feeding larvae.

Acknowledgments

This work is part of a Ph.D. thesis submitted to Laval University by the senior author who was financially supported by the Fundayacucho Program of Scholarships. We thank the technical and analytical assistance of the zooplankton staff of IOV-UDO (Instituto Oceanográfico de Venezuela, Universidad de Oriente), Domingo Figueroa and Rafael Briceño, and Caroline Berger for her work in the field survey, figure preparation and the processing of otoliths with Julie Paquet. Martin LLano, of the Metereological Station of Fundacion La Salle, kindly supplied the enviromental data. We thank Luis Trocolis, Jose Luis Fuentes, and Alfredo Gómez from de Edimar-UDO, Nueva Esparta, for laboratory support, Jose Bechara, Casimiro Quiñones, and Jean Paul Boulianne for advice and statistical support and Idelfonso Liñeros and Jesus Marcano for support and complementary information. This work was funded by grants from NSERC (Natural Sciences and Engineering Research Council of Canada) and FCAR (Fonds pour la Formation des Chercheurs et L'Aide à la Recherche, Québec, Canada) to Julian J. Dodson and GIROQ (Groupe Interuniversitaire de Recherches en Oceanographie du Québec) and by the Consejo de Investigación-Universidad de Oriente, proyecto LISA-92 (CI-5-019-00554/92).

Literature cited

Alvarez-Lajonchere, L.
1976. Contribución al estudio del ciclo de vida de *Mugil curema* Valenciennes (Pisces: Mugilidae). Cien. Ser. 8 Investig. Mar. (Havana) 28:3–130.
1980. Composición por especies y distribución de las postlarvas y juveniles de Lisas (Pisces, Mugilidae) en tunas de Zaza, Cuba. Rev. Investig. Mar. Cuba 1(2–3):28–60.
1982. The fecundity of mullet (Pisces, Mugilidae) from Cuban waters. J. Fish. Biol. 21:607–613.

Anderson, W. W.
1957. Early development, spawning, growth and occurrence of the white mullet (*Mugil curema*) along the south Atlantic

Coast of the United States. Fish. Bull.Wildl. Serv. 57(120): 415–425.

Angell, C.
1973. Algunos aspectos de la biologia de la lisa, *Mugil curema* Valenciennes, en aguas hipersalinas del nororiente de Venezuela. Contrib. Fund. La Salle. Cienc. Nat. 51: 223–238.

Bakun, A.
1986. Local retention of planktonic early life stages in tropical reef bank demersal systems: the role of vertically-structured hydrodynamic processes. *In* IOC/FAO workshop on recruitment in tropical coastal demersal communities, Ciudad del Carmen, Campeche, México. Workshop Rep. 44(suppl), p. 16–32.

Bakun, A., D. R. McLain, and F. V. Mayo.
1974. The mean annual cycle of coastal upwelling off western North America as observed from surface measurements. Fish. Bull. 72:843–844.

Bartsch, J., and R. Knust.
1994. Simulating the dispersion of vertically migrating sprat larvae (*Sprattus sprattus*) in the German Basin with a circulation and transport model system. Fish. Oceanogr. 3:92–105.

Blaber, S. J. M.
1987. Factor influencing recruitment and survival of mugilids in estuaries and coastal waters of Southeastern Africa. *In* Common strategies of anadromous and catadromous fishes (M. Dadswell, R. Klauda, C. Saunders, R. Rulifson, and J. Cooper, eds.), p. 507–518. Am. Fish. Soc.Symp. 1?

Blaber, S. J. M., and T. G. Blaber.
1980. Factors affecting the distribution of juvenile estuarine and inshore fish. J. Fish. Biol. 17:143–162.

Bowden, K. F.
1983. Physical oceanography of coastal waters, 302 p. Ellis Horwood Ltd., Chichester, UK.

Caldwell, D. K., and W. W. Anderson.
1959. Offshore occurrence of larval white mullet, *Mugil curema*, in the western Gulf of Mexico. Copeia 1959:252.

Campana, S. E., and J. D. Nielsen.
1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42:1014–1032.

Creutzberg, F., A. T. G. W. Eltink, and G. J. van Noort.
1978. The migration of plaice larvae *Pleuronectes platessa* in the western Wadden sea. *In* Physiology and behaviour of marine organisms (D. S. Lunsky and A. J. Berry, eds.), p. 243–251. Pergamon Press, New York, NY.

Cury, P., and C. Roy.
1989. Optimal environmental window and pelagic fish recruitment success in upwelling areas. Can. J. Fish. Aquat. Sci. 46:670–680,

Ditty, J. G., and R. F. Shaw.
1996. Spatial and temporal distribution of larval striped mullet (*Mugil cephalus*) and white mullet (*M. curema*, family: Mugilidae) in the northern Gulf of Mexico, with notes on mountain mullet, *Agonostomus monticola*. Bull. Mar. Sci. 59:271–288.

Etchevers, S. L.
1974. Fecundidad de la lisa (*Mugil curema* Valenciennes) en el Oriente de Venezuela. Bol. Científ. Téc. Ser. Recur. Mar. CIC UDO (Centro de Ingeniería y Computación, Universidad de Oriente) 1(1), 19 p.

Ferraz-Reyes, E.
1983. Estudio del fitoplancton de la Cuenca Tuy–Cariaco, Venezuela. Bol.Inst.Oceanogr.Venez.Univ.Oriente.22(1/2): 111–124.
1989. Influencia de los factores físicos en la distribución vertical de la biomasa fitoplanctónica en el Golfo de Cariaco (Venezuela). Bol. Inst. Oceanogr. Venez. Univ. Oriente. 28(1/2):47–56.

Ferraz-Reyes, E., E. Mandelli, and G. Reyes-Vasquez.
1987. Fitoplancton de la Laguna Grande del Obispo, Venezuela. Bol. Inst. Oceanogr. Venez. Univ. Oriente. 26(1/2): 111–124.

Franco, L.
1986. Biología y reproducción de la lisa, *Mugil curema* Valenciennes (Pisces: Mugilidae) en el Golfo de Cariaco, Venezuela. M.S. thesis, 103 p. Instituto Oceanografico de Venezuela, Univ. Oriente, Cumana, Venezuela.

Garcia, A., and G. Bustamante.
1981. Resultados preliminares del desove inducido de lisa (*Mugil curema* Valenciennes) en Cuba. Acad. Cienc. Cuba Inf. Cient.-Téc. 158:7–26.

Gines, H.
1972. Cartas pesqueras de Venezuela. Caracas, 328 p. Fundación La Salle de Ciencias Naturales. M.E.L.S.A., Madrid, Venezuela.

Gómez, A.
1983. Pigmentos clorofílicos, producción primaria y abundancia plantónica en el canal de entrada de la Laguna de la Restinga, Venezuela. Bol. Inst. Oceanogr. Univ. Oriente 22:43–64.

Gómez, A., and F. Cervigón.
1987. Perspectivas del cultivo de peces marinos en el Caribe Sur y noreste de Suramérica. Rev. Latinoam. Acuicult. 34: 40–50.

Heath, M. R.
1992. Field investigations of the early life stages of marine fish. Adv. Mar. Biol. 28:1–173.

Houde, E. D., S. A. Berkley, J. J. Klinovsky, and R. C. Schekter.
1976. Culture of larvae of the white mullet, *Mugil curema* Valenciennes. Aquaculture 8:365–370.

Ibañez-Aguirre, A. L.
1993. Coexistence de *Mugil cephalus* and *M. curema* in a coastal lagoon in the Gulf of Mexico. Mar. Biol. 42:959–961.

Jacot, A. P.
1920. Age, growth and scale characters of the mullets, *Mugil cephalus* and *Mugil curema*. Trans. Am. Microsc. Soc. 39: 199–229.

Jenkins, G. P., and H. M. A. May.
1994. Variation in settlement and larval duration of King George whiting, *Silloginodes punctata* (Sillaginidae), in Swan Bay, Victoria, Australia. Bull. Mar. Sci. 54(1): 281–296.

Johannes, R. E.
1978. Reproductive strategies of coastal marine fishes in the tropics. Environ. Biol. Fishes 3:65–84.

Jordan, A. R.
1993. Age, growth and back-calculated birthdate distribution of larval jack mackerel, *Trachurus declives* (Pisces: Carangidae) from eastern Tasmanian coastal waters. Aust. J. Mar. Freshw. Res. 45:19–33.

Li, C., S. Xueshen, Y. Feng, Y. Chunwu, and H. Ruidong.
1993. Daily growth increments in otoliths of mullet larva, *Mugil so-iuy* Basilewsky, and determination from field-collected ones. Oceanol. Limnol. Sin. 24(4):345–349.

McBride, R. S., and D. O. Conover.
1991. Recruitment of young-of-the-year bluefish *Pomatus saltatrix* to the New York Bight: variation in abundance and growth of spring- and summer-spawned cohorts. Mar. Ecol. Prog. Ser. 78:205–216.

Moore, R. H.
1974. General ecology, distribution and relative abundance

of *Mugil cephalus* and *Mugil curema* on the south Texas coast. Contr. Mar. Sci. 18:241–255.

Müller-Karger, F. E., C. R. McClain, T. R. Fisher, W. E. Esaias, and R. Varela.
1989. Pigment distribution in the Caribbean Sea: observations from space. Prog. Oceanogr. 23:23–64.

Neilson, J. D., and G. H. Geen.
1986. First-year growth rate of Sixes R. chinook salmon as infered from otoliths: effects on mortality and age at maturity. Trans. Am. Fish. Soc. 115:28–33.

Okuda, T., J. Benitez, and G. Cedeno.
1978. Caracteristicas hidrograficas del Golfo de Cariaco, Venezuela. Bol. Inst. Oceanogr. Venez. Univ. Oriente 17: 69–87.

Panella, G.
1971. Fish otoliths: daily growths layers and periodic patterns. Science. 173:1124–1127.

Radtke, R. L.
1984. Formation and structural composition of larval striped mullet otoliths. Trans. Am. Fish. Soc. 113:186–191.

Redding, M., and R. Patiño.
1993. Reproductive physiology. *In* Fish physiology (E. Evans, ed.), p. 503–534. CRC Press, Boca Raton, FL.

Rodriguez, C. L., and I. V. do Nacimiento.
1980. Estudio microscópico dos ovarios de *Mugil curema* Valenciennes, Brasil. *In* I simposio Brasileiro de aquacultura, Recife, 1978, p. 213–219. Academia Brasileira de Ciencias, Rio de Janeiro, Brazil.

Secor, D. H., J. M. Dean, and E. H. Laban.
1992. Otolith removal and preparation for microstructural examination. *In* Otolith microstructure examination and analysis (D. K. Stevenson and S. E. Campana, eds.), p. 9–57. Can. Spec. Publ. Fish. Aquat. Sci. 117.

Simonoff, J. S.
1996. Smoothing methods in statistics. Springer-Verlag, New York, NY.

Sinclair, M.
1988. Marine populations: an essay on population regulation and speciation, p. 252. Univ. Washington Press, Seattle, WA.

Sirois, P., and J. J. Dodson.
2000. Critical periods and growth-dependant survival of larvae of an estuarine fish, the rainbow smelt *Osmerus mordax*. Mar. Ecol. Prog. Ser. 203:233–245.

Struhsaker, P., and J. H. Uchiyama.
1976. Age and growth of the nehu, *Stolephorus purpureus* (Engraulidae), from the Hawaiian islands as indicated by daily growth increments in sagittae. Fish. Bull. 74:9–17.

Vieira, J. P.
1991. Juvenile mullets (Pisces: Mugilidae) in the estuary Lagoa dos Patos, RS, Brazil. Copeia 1991:409–418.

Wilson, K. H., and P. A. Larkin.
1980. Daily growth rings in the otoliths of juvenile sockeye salmon *Oncorhynchus nerka*. Can. J. Fish. Aquat. Sci. 37: 1495–1498.

Yañez-Arancibia, L. A.
1976. Observaciones sobre *Mugil curema* Valenciennes, en areas naturales de crianza, crianza, maduración, crecimiento, madurez y relaciones ecológicas. Ann. Cent. Cienc. Mar Limnol Univ. Nac. Autón. México 2:211–243.

Fecundity and spawning season of striped mullet (*Mugil cephalus* L.) in South Carolina estuaries*

Christopher J. McDonough
William A. Roumillat
Charles A. Wenner
Marine Resources Research Institute
South Carolina Department of Natural Resources
217 Fort Johnson Road
Charleston, South Carolina 29422-2559
E-mail address (for C. J. McDonough): mcdonoughc@mrd.dnr.state.sc.us

Abstract—Fecundity in striped mullet (*Mugil cephalus*) from South Carolina correlated highly with length and weight, but not with age. Oocyte counts ranged from 4.47×10^5 to 2.52×10^6 in 1998 for fish ranging in size from 331 mm to 600 mm total length, 2.13×10^5 to 3.89×10^6 in 1999 for fish ranging in size from 332 mm to 588 mm total length, and 3.89×10^5 to 3.01×10^6 in 2000 for fish ranging in size from 325 mm to 592 mm total length. The striped mullet in this study had a high degree of variability in the size-at-age relationship; this variability was indicative of varied growth rates and compounded the errors in estimating fecundity at age. The stronger relationship of fecundity to fish size allowed a much better predictive model for potential fecundity in striped mullet. By comparing fecundity with other measures of reproductive activity, such as the gonadosomatic index, histological examination, and the measurement of mean oocyte diameters, we determined that none of these methods by themselves were adequate to determine the extent of reproductive development. Histological examinations and oocyte diameter measurements revealed that fecundity counts could be made once developing oocytes reached 0.400 µm or larger. Striped mullet are isochronal spawners; therefore fecundity estimates for this species are easier to determine because oocytes develop at approximately the same rate upon reaching 400 µm. This uniform development made oocytes that were to be spawned easier to count. When fecundity counts were used in conjunction with histological examination, oocyte diameter measurements, and gonadosomatic index, a more complete measure of reproductive potential and the timing of the spawning season was possible. In addition, it was determined that striped mullet that recruit into South Carolina estuaries spawn from October through April.

Manuscript approved for publication 19 June 2003 by Scientific Editor.

Manuscript received 26 June 2003 at NMFS Scientific Publications Office.

Fish Bull. 101:822–834

The striped mullet (*Mugil cephalus* L.) is distributed circumglobally in tropical and semitropical waters between latitudes 42°N and 42°S (Thompson, 1963; Rossi et al., 1998). Even though considered a marine species, striped mullet are euryhaline and can be found year round throughout the full range of estuarine salinities in the southeastern United States (Jacot, 1920; Anderson, 1958). Striped mullet are a commercially important fish throughout the world sustaining both fisheries and aquaculture industries. In the southeastern United States (North Carolina and Florida) there are significant commercial fisheries for striped mullet, whereas in South Carolina and Georgia landings are more limited (NMFS[1]).

The primary fishery in most of these states is for "roe" mullet during the fall spawning migration. Throughout the rest of the year mullet are fished commercially for bait, if they are fished at all (Anderson, 1958). Striped mullet have a significant economic impact in the areas where they are more heavily fished by the commercial fisheries and the landings of this species from 1994 to 1998 yielded a landings (wholesale) value of 38.2 million dollars. Striped mullet are also one of the most important forage fishes that are found in the estuaries of the southeastern United States and represent a significant food source for upper-level piscivores (Wenner et al.[2]).

The biological features of striped mullet has been well documented (Jacot, 1920; Anderson, 1958; Thomson, 1963, 1966; Chubb et al., 1981), but much less information is available on the biological aspects of reproduction in the wild (Anderson, 1958; Stenger, 1959; Greeley et al., 1987; Render et al., 1995). There is a large body of work concerning striped mullet reproduction in aquaculture but many of these studies have used artificial manipulation of the reproductive cycle. Although the maturation process of oocytes may have been the same as that in wild striped mullet, the environment and conditions under which maturation occurs were artificial (Shehadeh et al., 1973a; Kuo et al., 1974; Pien and Liao, 1975, Kelly, 1990; Tamaru et al., 1994; Kuo, 1995). In addtion, despite the demonstrated ability to initiate reproduction (both in and out of season) for striped mullet, the majority of aquaculture studies have had to rely on wild fish for broodstock (Kuo et al., 1974; Pien and Liao, 1975; Kuo, 1995).

---

* Contribution 522 of the Marine Resources Institute, South Carolina Dept. of Natural Resources, Charleston, SC 29422-2559.

[1] National Marine Fisheries Service. 2001. Personal commun. Statistics and Economic Division, 1315 East-West Highway, Silver Spring, Md. 20910. http://www.st.nmfs.gov/st1/index.html.

[2] Wenner, C. A., W. A. Roumillat, J. E. Moran, M. B. Maddox, L. B. Daniel, and J. W. Smith. 1990. Investigations on the life history and population dynamics of marine recreational fishes in South Carolina, part 1. Completion report, project F-37, p. 3–13 and project F-31, p. 6–35. South Carolina Marine Resources Research Institute, P.O. Box 12559, Charleston, SC 29422

In the southeastern United States the spawning season lasts from two to five months depending on the coastal area involved (Jacot, 1920; Broadhead, 1956; Anderson, 1958; Arnold and Thompson, 1958; Stenger, 1959; Dindo and MacGregor, 1981; Greeley et al., 1987; Render et al., 1995; Hettler et al., 1997). Striped mullet are considered isochronal spawning fishes (Greeley et al., 1987; Render et al., 1995), i.e. they have synchronous gamete development and individuals spawn all their reproductive material at once or in batches over a very short time period (days, as opposed to weeks). There have been limited observations of offshore spawning activity (Arnold and Thompson, 1958), and few examples of eggs and larvae collected offshore (Anderson, 1958; Finucane et al., 1978; Collins and Stender, 1989). Collins and Stender (1989) concluded that striped mullet spawn in and around the edge of the continental shelf off the coasts of North Carolina, South Carolina, Georgia, and the east coast of Florida (an area often referred to as the South Atlantic Bight) and have a protracted spawning season from October to April. This spawning season contrasts with that estimated by most other studies (Jacot, 1920; Broadhead,1956; Anderson, 1958; Arnold and Thompson, 1958; Stenger, 1959; Dindo and MacGregor, 1981; Greeley et al., 1987; Render et al., 1995; Hettler et al., 1997). These studies based their estimates on the reproductive condition of migrating adults and the subsequent recruitment of juvenile fish back into coastal estuaries—not on actual data on the offshore presence of striped mullet larvae. Female mullet have been shown to mature at two to three years of age at a size range from 230 mm to 350 mm standard length (Thomson, 1951, 1963; Greeley et al., 1987). Determination of spawning activity in mullet has been estimated by using gonadosomatic indices (Dindo and MacGregor, 1981; Render et al., 1995), examination of oocyte size and maturity stage (Stenger, 1959; Greeley et al., 1987), and by the presence of enlarged, developing ovaries in migrating fish (Jacot, 1920; Anderson, 1958).

The purpose of the present study was to develop size- or age-related estimates of fecundity in striped mullet from South Carolina estuaries. These fecundity estimates were determined in order to develop models for estimating potential fecundity from catch data, such as length and weight data. In addition, other indicators of reproductive activity such as gonadosomatic indices and oocyte size were examined to provide information on the duration of the spawning season. Potential fecundity estimates can give a barometer of reproductive potential based on morphological information from catch curve data and size-frequency distributions.

Materials and methods

Striped mullet were collected monthly from January 1998 through December 2000 by using a randomly stratified sampling regime within three different estuarine systems along the South Carolina coast: (Ashepoo-Combahee-Edisto (ACE) Basin, Charleston Harbor, and the Cape Romain estuary, Fig. 1). The female striped mullet used for fecundity estimates were collected from October through February each year because these were the only months when fecund females were present. Fecund female striped mullet were defined as specimens in the tertiary stage of vitellogenesis with oocyte diameters greater than 400 μm and that had gonadosomatic indices greater than 5.0. The vitellogenic stage for each specimen used in the fecundity estimates was confirmed histologically. Fish were captured during daylight ebbing tides with water levels ranging from 0.3 to 2.0 meters, and the majority (67.8%) were caught during late ebb. The fish were primarily caught with a 184-m trammel net with an outside stretch mesh of 350 mm and an inside stretch mesh of 63.5 mm, although a few of the 1999 samples (5) and the 2000 samples (3) were obtained by using a cast net 1.8 m in diameter and equipped with 10-mm mesh. Eight fecund females were also captured by using an electroshock boat in the freshwater and low-salinity areas of the Cooper River (one of the three rivers that make up the Charleston Harbor Estuary) in October 2000. The areas included in the present study have been sampled on a monthly basis since 1991 with trammel nets by the Inshore Fisheries Group of the South Carolina Department of Natural Resources as part of a gamefish monitoring program. During this period reproductively developing striped mullet of both sexes were generally observed from October through February and were presumably heading offshore to spawn (Jacot, 1920; Anderson, 1958; Arnold and Thompson, 1958). Male striped mullet that were reproductively developed were easy to discern because they were usually leaking milt and were not analyzed further. All other fish were brought back to the laboratory and eviscerated in order to determine sex and to collect ovaries for reproductive analysis. All of the samples were kept on ice and were generally examined within twenty-four hours of capture.

Standard morphometric measurements included total length (TL) in mm, fork length in mm (FL), and standard length in mm (SL), total weight (TW) in grams, ovary weight (OW) in grams, sex, and maturity. Body weight (BW) was calculated as total weight minus ovary weight ($BW=TW-OW$). Saggital otoliths were removed for aging. A small section of the posterior end of the ovaries, at the junction of the two lobes, was also removed for histological examination. The whole ovaries were fixed in 10% seawater-buffered formalin and the histological sample was fixed in 10% neutral-buffered formalin. Histological samples were processed by using standard procedures for paraffin embedding and sectioning (Humason, 1967). The sections were dried on slides and stained by using standard haematoxylin and eosin-Y staining techniques (Humason, 1967). Examination of the histological sections for maturity stage was done by using a compound microscope at 100× magnification. Each histological section was evaluated by two separate readers to determine agreement on maturity stage. If there was a discrepancy in maturity staging for any specimen, the discrepancy was either resolved by the two readers or that specimen was not used in the analysis. There were no discrepancies between readers on any of the reproductively developing females. Maturity was assessed according to a modified version of the schedule used by Wenner et al. (1986) adapted to work with isochronal

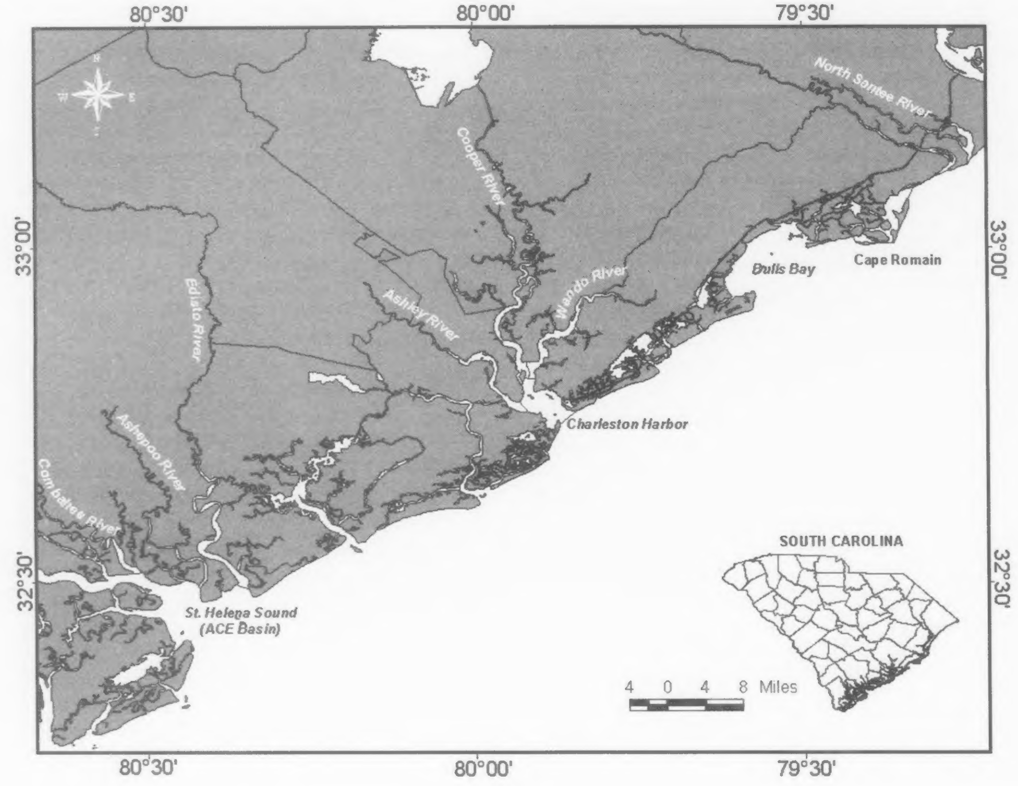


Figure 1

The South Carolina coast with the locations of estuaries and major river basins where striped mullet were collected from 1998 to 2000.

spawning fish, as well as previous models of reproductive development (Stenger, 1959; Wallace and Selman, 1981) (Table 1). This evaluation method was based on identification of morphological characteristics evident in histological sections. Each specimen was evaluated by two (in some cases three) readers and discrepancies between readers were either resolved or the specimen was excluded from the analysis.

A gonadosomatic index (GSI) was calculated for each specimen following the method of Render et al. (1995) where GSI was expressed as gonad weight (GW) divided by body weight (BW) such that

$$GSI = (GW/BW) \times 100.$$

The GSI values of the fecundity specimens were compared among the three sampling years, as well as with GSI values for female striped mullet collected during the rest of the year that were mature but not reproductively active.

Fecundity determinations were made from a total of 129 advanced-stage developing ovaries: 50 from 1998, 37 from 1999, and 42 from 2000. All of the ovaries were determined by histological examinations and criteria outlined in Table 1 to be actively vitellogenic, having tertiary yolk-stage oocytes. All oocytes counted for fecundity were 400 μm or larger and in the tertiary yolk stage (Pien and Liao, 1975) (Fig. 2). This 400-μm threshold has also been used in other studies of oocyte development in striped mullet for determining the point at which the oocytes that would be spawned during that year were identifiable (Shehadeh et al., 1973a). Unlike other species (particularly batch spawners), where fecundity counts should ideally be conducted by using hydrated oocytes only, the striped mullet oocytes used for fecundity counts were still presumably several weeks from hydration and spawning. However, because mullet are synchronous spawners, it is relatively easy to distinguish the developing oocytes from the undeveloped ones because of the drastic difference in size between the two, as well as by the uniformity in size of the developing oocytes once they reach 400 μm.

Fecundity was estimated by using a modified gravimetric method. The fixed whole ovary was patted dry and reweighed. The ovarian lobes were divided into four discrete regions along each lobe's longitudinal axis and three subsamples (chosen at random) were taken between the two lobes and preserved in 50% isopropyl until oocyte counts could be conducted. The subsamples ranged in weight from 0.025 g to 0.033 g. The subsamples from each specimen

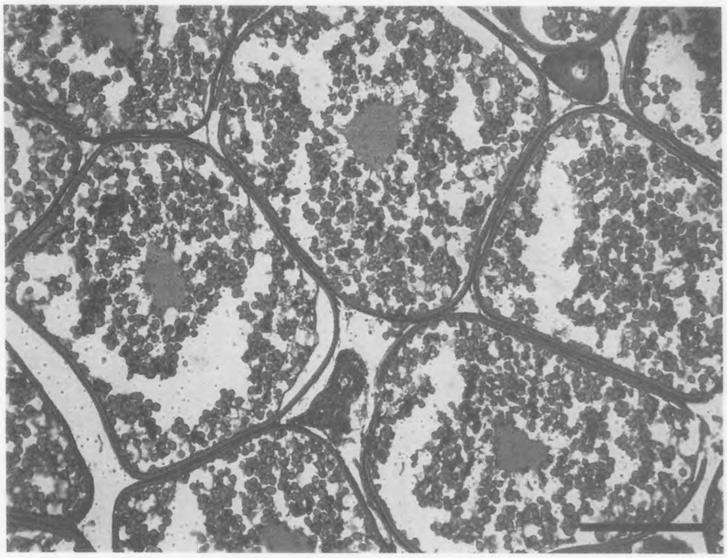


Figure 2

A striped mullet oocyte in the tertiary yolk stage of vitellogenesis. Scale bar = 250 μm.

Table 1

Histological criteria used to determine reproductive stage in female striped mullet (*Mugil cephalus*) once sexual differentiation has occurred (Wenner et al.: see footnote 2 in the general text).

Reproductive stage	Description
1. Immature	Inactive ovary with previtellogenic oocytes and no evidence of atresia. Oocytes are < 80 μm, lamellae still contain somatic and connective tissue bundles. Ovary wall is very thin (one or two cell layers).
2. Developing	Developing ovary have enlarged oocytes generally greater than 120 μm in size. Cortical alveoli are present and actual vitellogenesis occurs after oocytes reach 180 μm in size and continue to increase in size. Abundant yolk globules with oocytes reaching a size of >600 μm.
3. Running ripe	Completion of yolk coalescence and hydration in most oocytes.
4. Atretic or Spent	More than 30% of developed oocytes undergoing the atretic process.
5. Inactive or Resting	Previtellogenic oocytes only but traces of atresia possible. In comparison to immature females, most oocytes are >80 μm, lamellae have some muscle and connective tissue bundles. Lamellae are larger, have moore oocytes, and are elongated. A thicker ovarian wall with blood vessels, muscle, and nerve tissue.

were then teased apart. After separation, the oocytes were spread out on a Bogorov tray and counts of oocytes, greater than 400 μm, were made by using a dissecting microscope at 12× magnification. Each subsample was counted twice and counts were averaged. A third count was performed if the first two counts differed by more than 10%. Oocyte density was calculated by dividing the mean number of oocytes by the mean weight of all three subsamples for each specimen. The oocyte density was then used to calculate the total oocyte number for each ovary, or individual fecundity, by multiplying mean oocyte density by whole ovary weight.

In order to determine mean oocyte diameter for each specimen, 20–30 oocytes were removed from each counted subsample and grouped together in a petri dish. Each oocyte was then measured along the longest axis by using Optimas^{TM} Image Analysis software (version 6, Media Cybernetics, Bothell,WA). Mean oocyte diameter was calculated as the average of all measurements for each

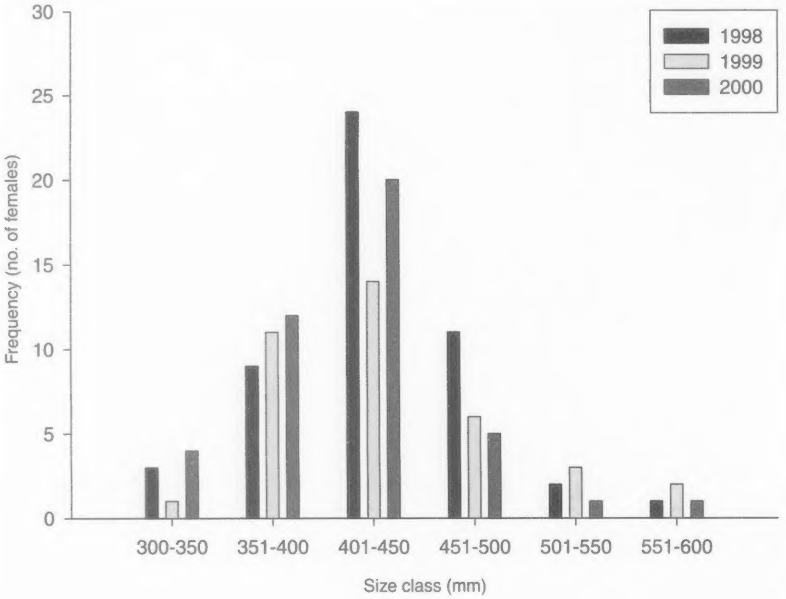


Figure 3

Size-frequency distribution of female striped mullet used in fecundity determinations from South Carolina estuaries from 1998 to 2000. *n* (number of fish in sample) = 50 fish for 1998, *n* = 37 for 1999, and *n* = 42 for 2000.

subsample. The overall mean oocyte diameter for each specimen resulted from the calculated average of the means of the three subsamples. Measurements were not made on fresh oocytes but shrinkage was estimated from the amount of whole ovary shrinkage because fresh ovary weight and preserved (in 10% formalin) ovary weight were known. The estimated unpreserved oocyte diameter was determined by multiplying the preserved oocyte diameter by 1 and adding the percentage of ovary shrinkage. The difference in the preserved oocyte diameter and the estimated fresh oocyte diameter was then compared by using a paired *t*-test to determine if there was a significant difference between preserved and unpreserved oocyte diameters.

Age was determined by using the left sagittal otolith, which was embedded in epoxy resin. A 0.5-mm transverse section encompassing the otolith core was cut with an Isomet low-speed saw with diamond wafering blades. The thin section of the otolith was embedded in epoxy and observed with a dissection microscope at the magnification appropriate for the otolith's size. Age was recorded as the number of rings (annular bands) present. The otoliths were initially aged by one reader. A second reader then evaluated a subsample of specimens from 1998 and 2000 and all the otoliths from 1999. Ages were validated by the percentage of agreement between the two age determinations, an analysis of variance (ANOVA) between the two groups of ages, and a paired *t*-test comparing the means and variances of the two groups (Campana et al., 1995).

Results

Fecund female striped mullet (again, defined as those females with ovaries containing oocytes >400 μm in the tertiary stage of vitellogenesis) were collected from late October through February; most of the specimens were caught in November and December for all three years. Size-frequency distributions did not vary over the three years of the study (Fig. 3). Fewer fish (*n*=37) were taken in 1999 versus 1998 (*n*=50) and 2000 (*n*=42). The most abundant size class for each year was that from 401 to 450 mm. In the overall size-frequency distributions, fecund females made up greater than 44% of those fish larger than 400 mm in 1998 and comprised all of the specimens over 500 mm. In 1999 the fecund females made up 12.5% of fish in the 401–450 mm size range, 39% of the fish in the 451–500 mm size range, and 100% of the fish in the size classes over 500 mm. In 2000, fecund females made up 17.7% of the 401–450 mm size class, 35.7% in the 451–500 mm size range and, like 1998 and 1999, all of the specimens over 500 mm.

The majority of females used in our fecundity study were 3 or 4 years old (Fig. 4), accounting for 80.0% of the specimens in 1998 and 73.3% in 1999. However, the age distribution in 2000 showed that the frequency of 2-, 3-, and 4-year-olds was the same and that these three ages made up 82.0% of the fecund fish sampled that year. Three-year-old fish made up the largest single group in 1998 and 1999. The age determined from the otoliths was validated as part

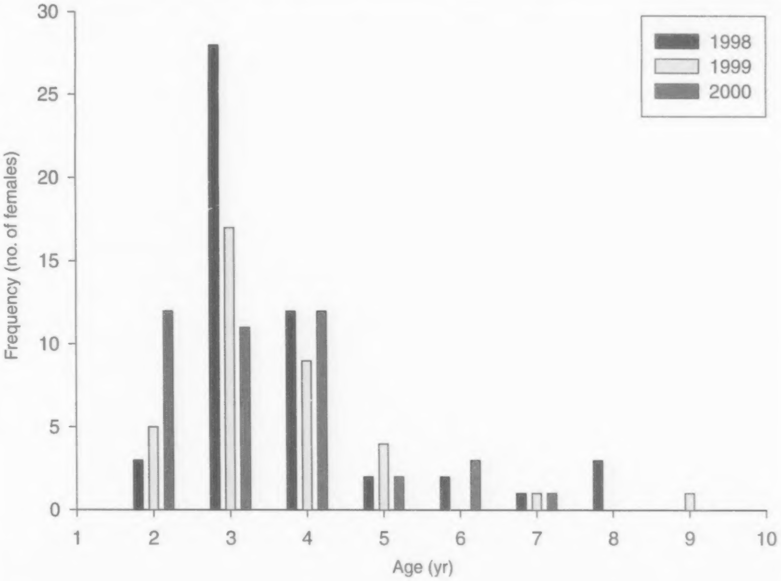


Figure 4

Age-frequency distribution of fecund striped mullet in South Carolina estuaries from 1998 to 2000. *n* (number of fish in sample) = 50 fish for 1998, *n* = 37 for 1999, and *n* = 42 for 2000.

of another study where size and age structure of striped mullet in South Carolina was examined (Wenner and McDonough, unpubl. data[3]). A comparison of multiple readings of the same group of otoliths assessed aging precision. One year (1999) was chosen at random and all of the otoliths (n=1234) from that year were aged by a second reader. The ages of the two independent determinations were then compared by using a one-way ANOVA and a t-test. The variance statistic was 2.78 for the original ages and 2.81 for the second age reads, which were not significantly different (P=0.001) and both had almost identical normalized residuals. Overall, there was an 83.4% agreement on ages between readers. The results from the ANOVA (F=1555.0, df=10, P=0.000) and the t-test (t=2.898, df=1233, significance [2-tailed]=0.004) both confirmed that there was no significant difference between the separate age determinations. Therefore, the age recorded by the first reader for all specimens was used in the analysis.

The length-weight relationship for fecund female striped mullet was compared by using a linear regression of (natural) log-transformed body weight against total length to see if there were any differences between years. The regression coefficients from each year were compared by using a test of significance between more than two slopes (Zar, 1984). The weight measurement used was total body weight minus gonad weight (TW–OW=BW) because ovary weight had a considerable influence on total body weight in the fecund specimens (GSI values of 7.7 to 27.7). There was no significant difference in the total length to Ln body weight regressions between different years (F=9.22, P=0.001, df=129). Because there was little difference in the regressions between years and in order to increase sample size, data from all three years were combined to obtain the overall total-length to Ln-body-weight relationship of Ln $BW = -11.1 + 2.92\ (TL)$ (Fig. 5). In contrast, the length and body-weight-at-age relationships were highly variable; a wide range of sizes occurred in the 2-, 3-, and 4-year age classes (Fig. 6). The high degree of variability was also exacerbated by the smaller number of fish age 5 or older.

The gonadosomatic index (GSI) for fecund mullet ranged from 7.7 to 27.5 in 1998, 9.3 to 27.7 in 1999, and 9.5 to 26.6 in 2000. In contrast, the GSI for mature females that were not undergoing any reproductive development ranged from almost zero to 4 for all three years of the study. The relationship of GSI to size (TL or BW) was not very strong in any year. However, GSI was positively correlated (P=0.01) with oocyte diameter and negatively correlated with oocyte density (Table 2) because of the inverse relationship of oocyte density and oocyte diameter. The correlation coefficient for GSI and age were very close to zero and slightly negative (Table 2).

Mean GSI by month for males and females (Fig. 7) increased from October through April, peaking in November–

---

[3] Wenner, C. A., and C. J. McDonough. 2001. Cooperative research on the biology and assessment of nearshore and estuarine fishes along the southeast coast of the U.S. Part IV: Striped mullet, *Mugil cephalus*. Final rep, Grant no. NA77FF0550, 82 p. Marine Resources Research Institute, South Carolina Dept. of Natural Resources, P.O. Box 12559 Charleston, S.C. 29422-2559.

December. The duration of the reproductive season, as evidenced by advanced reproductive condition determined by the GSI, was also confirmed from histological assessments of maturity stages for all gonads collected during this time period (not just those used for the fecundity study) which indicated that reproductively developing males were present August through February, whereas reproductively developing females were present August through April (Fig. 8).

There was no significant difference in oocyte density among subsamples taken from different areas of the ovary lobe. This result was obtained by using an ANOVA of oocyte densities between the four divided areas of the ovary lobes where subsamples were taken (F=0.421, df=3). This analysis allowed us to accept the assumption that oocytes were equally distributed throughout the ovary lobes, which provided validation for the random sampling of oocytes from different areas of the lobe in order to determine individual fecundity.

The regression of individual fecundity with total length (TL) was not a linear relationship, whereas the regression of fecundity on body weight (BW) was linear. Therefore, the comparisons of individual fecundity to total length (TL) and body weight (BW) were made by using both the raw data and the data with natural log transformations. The range of specimen total lengths was 291 to 600 mm in 1998, 332 to 588 mm in 1999, and 325 to 592 mm in 2000 and for body weight 242 to 2149 g in 1998, 335 to 2008 g in 1999, and 284 to 2144 g in 2000. Mean fecundity, compared between years with a two sample t-test, was significantly different between 1999 and 2000 (t=0.019, df=78, P=0.985) but was not significantly different between 1998 and 1999 (t=0.974, df=86, P=0.336)

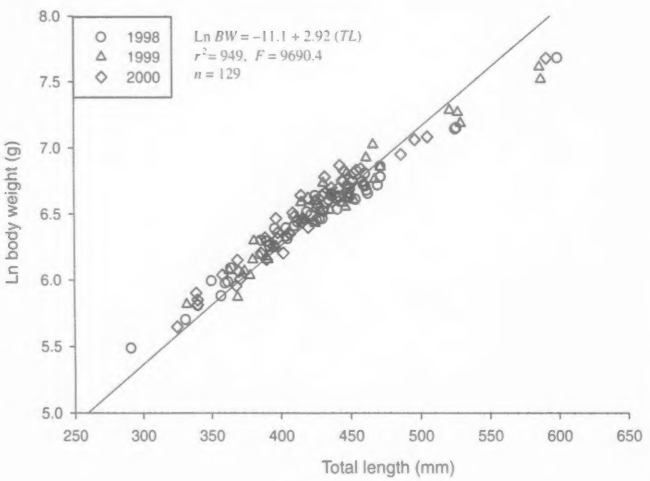


Figure 5

Regression analysis of log-transformed (Ln) body weight on total length for fecund striped mullet in South Carolina estuaries from 1998 to 2000. n (number of fish in sample) = 129.

Table 2

Pearson correlation coefficients, with significance values, for the morphological variables and fecundity of striped mullet in South Carolina estuaries from 1998 to 2000. n (number of fish in sample) = 129. TL = total length, BW = body weight, ODM = oocyte diameter, ODN = oocyte density, GSI = gonadosomatic index, FEC = fecundity.

		Age	TL	BW	ODM	ODN	GSI	FEC
Age	Pearson correlation	1.000						
	significance (2-tailed)							
TL	Pearson correlation	0.117	1.000					
	significance (2-tailed)	0.186						
BW	Pearson correlation	0.164	0.951**	1.000				
	significance (2-tailed)	0.062	0.000					
ODM	Pearson correlation	0.004	0.101	0.029	1.000			
	significance (2-tailed)	0.964	0.254	0.741				
ODN	Pearson correlation		−0.050	−0.008	0.095	−0.628**	1.000	
	significance (2-tailed)	0.575	0.927	0.282	0.000			
GSI	Pearson correlation	−0.029	0.128	−0.006	0.543**	−0.645**	1.000	
	significance (2-tailed)	0.746	0.147	0.947	0.000	0.000		
FEC	Pearson correlation	0.113	0.892**	0.888**	0.059	0.142	0.260**	1.000
	significance (2-tailed)	0.200	0.000	0.000	0.504	0.105	0.003	

** Correlation is significant at the 0.01 level (2-tailed).

and between 1998 and 2000 (t=1.368, df=92, P=0.179). However, given that the mean fecundity was 1.18 million oocytes in 1998, 1.16 million oocytes in 1999, and 1.09 million oocytes in 2000, the difference in mean fecundity between 1999 and 2000 was probably not biologically significant. It was determined that data could be pooled across years for several reasons. The coefficients of determination for each year indicated that there was a similarly strong relationship of fecundity to total length and body weight in all three years and the coefficients of variation for each year (0.408 for 1998, 0.594 for 1999, and 0.457 for 2000 at P=0.001) were not significantly different. By pooling the data from all three years we were able to determine two models of potential fecundity based on total length (TL) and body weight (BW) (Fig. 9):

$$\text{Ln } \mathit{Fecundity} = -6.86 + 3.42(\text{Ln } \mathit{Total\ Length}) \quad [r^2=0.803, F=527.2, \text{df}=129]$$

$$\text{Ln } \mathit{Fecundity} = 6.95 + 1.05(\text{Ln } \mathit{Body\ Weight}) \quad [r^2=0.804, F=530.6, \text{df}=129].$$

The r^2 values for untransformed data were very close to the values obtained with transformed data (r^2=0.795, F=502.9 for fecundity on total length [TL] and r^2=0.787, F=479.4 for fecundity on body weight [BW]). The high r^2 values, as well as the high correlation coefficients between fecundity and total length and body weight (Table 2) indicated that potential fecundity was size dependent.

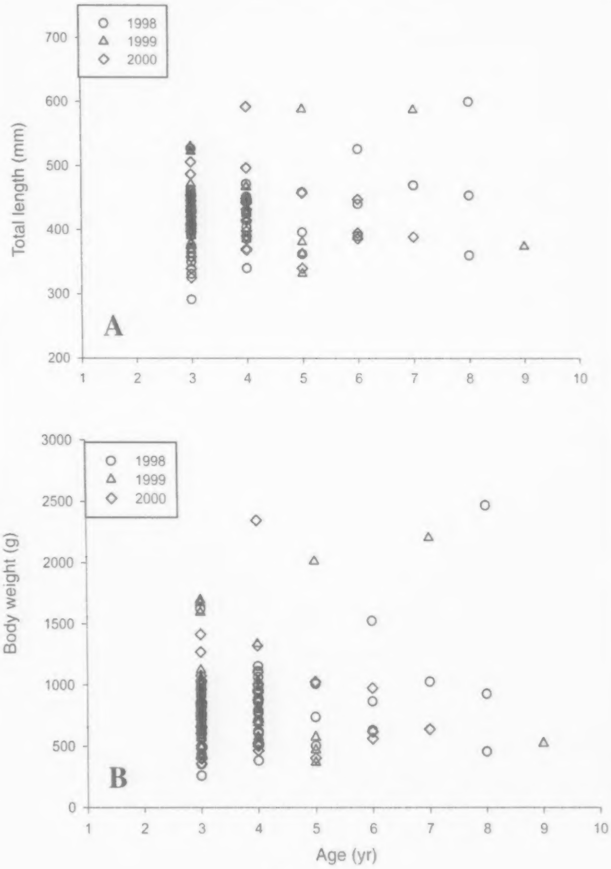


Figure 6

Relationship of total length (**A**) and body weight (**B**) to age for fecund striped mullet in South Carolina estuaries from 1998 to 2000. n (number of fish in sample) = 129.

Unlike fecundity, oocyte density did not change with size (TL or BW) in 1998 and 1999 and increased with size in 2000. The increase in density in 2000 was due to a group of fish captured in freshwater in October having relatively low GSIs and high densities of oocytes that also happened to be some of the largest fish captured that year. Oocyte density was negatively correlated with GSI (Table 2), and thus indicated that increasing GSI resulted in lower oocyte densities.

There was not a high degree of variability in oocyte diameter over the entire size range for the three years of the study. Oocyte diameter did appear to increase with age in 2000 and remained stable for 1998 and 1999. However, the increase in oocyte diameter in 2000 was not statistically significant.

In a comparison of mean oocyte diameter in each size class (total length) by month of capture, the data for all three years were pooled in order to obtain adequate representation in each month. Oocyte size ranged from 463 to 682 μm and the mean size was 596 μm. The largest mean oocyte diameters were found in specimens captured in January and February. Specimens were captured during the months of November and December for all size classes and there was an increase in oocyte diameter with each progressive month. In particular, females in the 400–500 mm size range (which represented the largest number of specimens) were examined and there was a progression of increasing oocyte diameter with month of capture through the reproductive season.

The increase in oocyte size, as the reproductive season progressed, was more apparent when mean oocyte diameter by month for each year separately was examined. Specimens were collected from October through February in 1998, November through January in 1999, and October through December in 2000. Equal effort was made during all of these months of each year to capture specimens, but they were not always available for capture. There was an increase in mean oocyte size per month as the spawning season progressed in all three years (Fig. 10). Even though the largest oocyte size measured was 682 μm, this measurement was that of a preserved oocyte. If we factor in a mean shrinkage of 4%, maximum oocyte size becomes 709 μm.

The paired *t*-test showed no significant difference between the preserved oocytes and the predicted size of fresh oocytes (t=–26.2, df=128, P=0.000).

Discussion

Fecundity in striped mullet from South Carolina correlated highly with length and weight, but not with age. Oocyte counts ranged from 4.47×10^5 to 2.52×10^6 in 1998 for fish ranging in total length from 331 mm to 600 mm, 2.13×10^5 to 3.89×10^6 in 1999 for fish ranging in total length from 332 mm to 588 mm, and 3.89×10^5 to 3.01×10^6 in 2000 for fish ranging in total length from 325 mm to 592 mm. These fecundity levels correspond with general fecundity levels (2.0×10^5 to 14.0×10^6) found in striped mullet in northeast Florida (Greeley et al., 1987), the Gulf Coast (Render et al., 1995) as well as studies in Europe and Asia (review by Alvarez-Lajonchere, 1982). One marked difference in fecundity between the present study and some in the literature was the difference in oocyte density. Render et al. (1995) found densities ranging from 798 to 2616 oocytes/g ovary weight, whereas densities in the present study ranged from 1710 to 14,817 oocytes/g ovary weight. However, although fecundity increased with both total length and body weight in 1998 and 1999, densities did not. The lower oocyte densities in the larger fish were most likely indicative of larger oocytes. This feature is common in both synchronous and asynchronous spawning fishes (Greeley et al., 1987; Render et al., 1995; Fox and Crivelli, 1998; DeMartini and Lau, 1999). Because total length and body weight were more highly correlated with increased fecundity, the larger specimens would have made a greater individual reproductive contribution during any given spawning season (Korhola et al., 1996; Kaunda-Arara and Ntiba, 1997; DeMartini and Lau, 1999). Making estimates of potential fecundity from age alone was difficult because of the variability in the size-at-age relationship; however, fecundity estimates from total length or body weight appeared more reliable and reflected values closer to the fecundity levels observed in the present study.

Previous studies have reported that female mullet become reproductively mature at three years of age (Thomson, 1951; 1963; Stenger, 1959; Chubb et al., 1981; Render et al., 1995). Greely et al. (1987) suggested that fecundity specimens collected in northeastern Florida were as young as two years at maturity. But, Greely et al. (1987) did not age the striped mullet used in their study and inferred age from a size-at-age growth schedule (Thomson, 1966). In the present study, two-year-olds made up only a small percentage of the fecund fish in 1998 and 1999. However, the results were different for the 2000 specimens in that there was an almost equal distribution in the age frequency of two-, three-, and four-year- olds. However, three- and four-year-olds made up the greatest percentage of females with advanced ovaries. Maturing fish were those undergoing active vitellogenic development and were generally captured in and around inlets or estuaries. Our study would suggest that female striped mullet reach 50% maturity at age 2 and 100% maturity at age 3. Three- and four-year-olds made up the majority of reproductively advanced fish in all years, whereas less abundant older fish made less of a contribution towards total reproductive effort.

There are several possible explanations accounting for the wide age distribution in maturity stages; the most likely is that fecundity is size related despite the highly variable growth rates and the widely ranging size at age in adult striped mullet. Size at maturity has been found to range widely from 230 mm standard length (Thomson, 1963; Greeley et al., 1987; Tamaru et al., 1994) up to 410 mm standard length (Thomson, 1963; 1966; Chubb et al., 1981) for two- and three-year-old fish. The lower end of this size range agrees quite readily with the lower size range (291 mm TL=239 mm SL) found in our study. In a concurrent study of maturity schedules related to size and age in South Carolina striped mullet (McDonough, unpubl. data), male striped mullet were found to mature at two years of age and as small as 250 mm total length (190 mm standard length). Other species of mullet have been shown to mature over a wide range of sizes. The Pacific mullet (Mugil so-iuy) becomes mature upon reaching approximately 430 mm total length (Okumus and Bascinar, 1997).

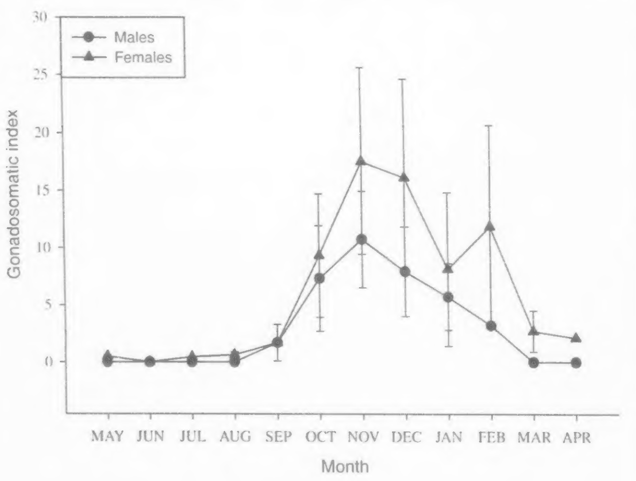


Figure 7

Mean gonadosomatic index value by month for male and female striped mullet from South Carolina estuaries from 1998 to 2000. *n* (number of fish in sample) = 455.

Monthly GSI levels clearly showed that the time period of reproductive activity is from October through April. Female striped mullet in all reproductive developmental stages were observed during the course of our sampling, with the exception of stage-3 (hydrated oocytes) females and females with recently spawned ovaries (characterized by the presence of postovulatory follicles). Atretic ovaries were observed from December through May. There were no postovulatory follicles observed, indicating that any atretic ovaries were not from recently spawned fish. The fish with atretic ovaries were characteristically emaciated for their size (TL and BW) and were most common from January through March. The presence of females with atretic ovaries starting in December is strong evidence that spawning occurred in November, if not earlier, and females with atretic ovaries caught as late as May demonstrate that spawning may still occur as late as April. Additional evidence for the October through April spawning period has also been shown in backcalculated birth dates for juvenile striped mullet by daily growth increments (McDonough and Wenner, 2003). This evidence supports the concept of offshore spawning in striped mullet and a yet undetermined time period required for moving from the estuaries to the spawning areas and for returning again to the estuaries. Other authors have come to the same conclusion from similar evidence in estuaries throughout the southeast (Jacot, 1920; Broadhead, 1956; Anderson, 1958; Stenger, 1959; Shireman, 1975; Dindo and MacGregor, 1981; Greeley et al., 1987; Render et al., 1995; Hettler et al., 1997). All of the fecundity specimens were caught from October through February when the mean monthly GSI was highest. Pien and Liao (1975) found that mullet oocytes reached a hydrated size of 900 to 1000 μm. The size of oocytes used for fecundity counts in the present study ranged from 463 to 682 μm. The maximum size of oocytes in the tertiary stage of vitellogenesis from our study was 600 μm or greater. This result agrees with those of previous studies where the maximum size of oocytes prior to either hydration or atresia (if spawning did not occur) ranged from 600 to 700 μm (Shehadeh et al., 1973b; Kuo et al., 1974). There was no evidence of prespawning atresia in any of the specimens used for fecundity estimates.

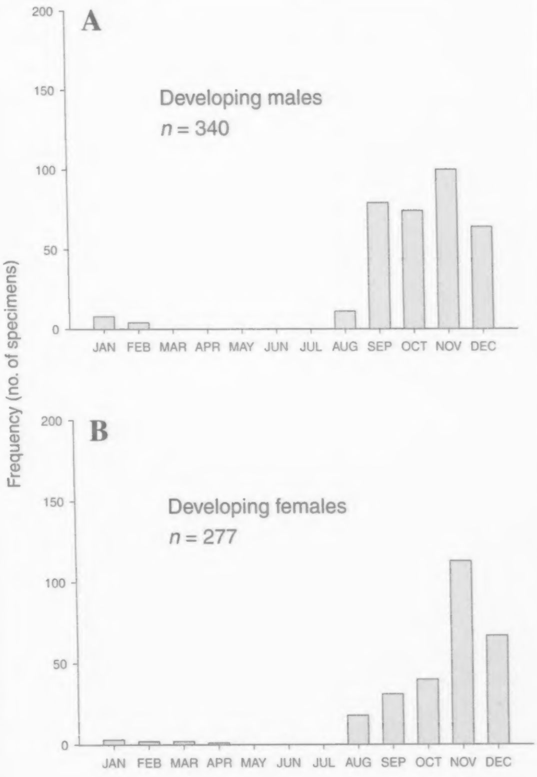


Figure 8

Frequency distribution of reproductively developing male (**A**) and female (**B**) striped mullet by month in South Carolina estuaries from 1998 to 2000.

The appropriateness of using a GSI alone to determine the level of reproductive development has been questioned, particularly for serial or asynchronous spawning fishes (De Vlaming et al., 1980; Hunter and Macewicz, 1985). Striped mullet can have a wide range of GSI values that range from practically zero to over thirty (Render et al., 1995). The GSI range for females in our study ranged from almost zero to 27.7. Because of the high variability in GSI with size, it does not appear appropriate to use GSI alone in order to assess reproductive development in striped mullet. When used in conjunction with histological analysis and mean oocyte diameter of tertiary-stage oocytes, GSI does provide excellent supporting evidence of reproductive schedules and spawning season duration. GSI is probably more appropriately used for isochronal spawning fishes than for serial spawning fishes because of the uniform development of oocytes in the former. However, it is still difficult to meet all the basic assumptions of the GSI index as given by De Vlaming et al. (1980) because of the high variability of GSI with size. Another technique that has been used in aquaculture situations to assess maturity and sex involves the use of a cannula to remove oocytes from the ovaries of live fish which are then evaluated (Shehadeh, et al., 1973a; Kuo et al., 1974). This technique, although useful for determining sex and the extent or stage of reproductive development, would be inappropriate for estimating potential fecundity.

Historically, reproductively developing mullet have been found in southeast United States waters from November through December (Jacot, 1920; Anderson, 1958; Stenger, 1959). During our study, reproductively developing mullet were caught in the Charleston Harbor Estuary from October through February. Gonad development of these fish was discernible through gross morphological observation, and histological sections showed that vitellogenesis was well underway. Other studies have shown that previtellogenic oocytes were usually less than 160 μm and that the onset of vitellogenesis began when the oocytes reached a size of 180 µm (Dindo and MacGregor, 1981; Greeley et al., 1987; Tamaru et al., 1994; Render et al., 1995). Developing individuals caught during our study that were not used for fecundity counts had less-developed ovaries, GSI values less than 7, and mean oocyte diameters less than

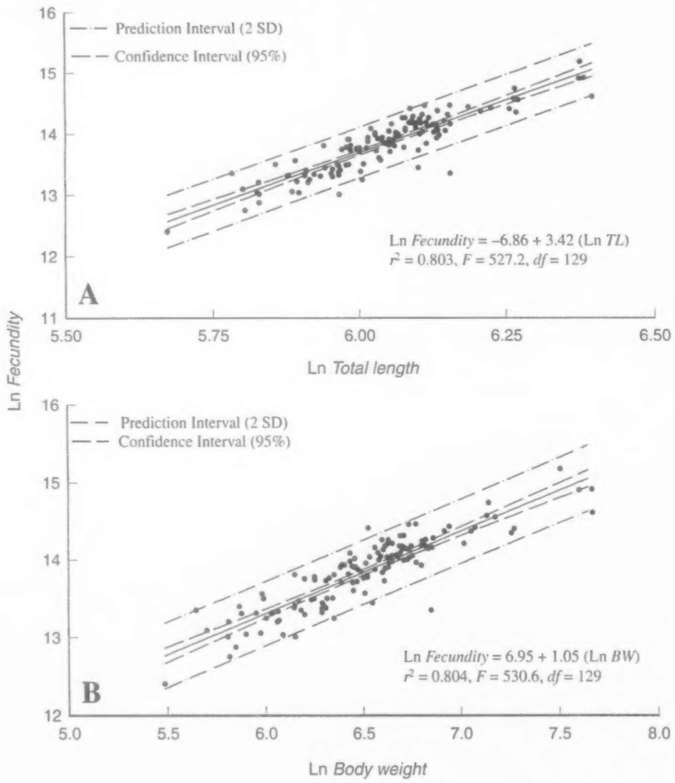


Figure 9

Regression of the natural log (Ln) transformation of individual fecundity on total length (**A**) and body weight (**B**) for striped mullet combined data, 1998 to 2000, from South Carolina estuaries. n (number of fish in sample) = 129.

350 μm. The vitellogenic activity in these ovaries was still in the primary and secondary stages. These specimens could not be used for fecundity counts because not all of the oocytes destined for that year's spawning batch had developed enough to be separable from the smaller oocytes that would not develop. Once the developing oocytes reached a size of 400 μm or larger, they became more uniform in size and in appearance and it was obvious which oocytes would constitute that year's spawn. At that point, fecundity could be determined more accurately because all the oocytes to be counted were significantly and equally larger. This point in particular is important in that it makes fecundity estimates from nonhydrated oocytes more accurate for isochronal spawning fishes, such as striped mullet. Fecundity estimates made in fishes that are batch-spawners should only be made from hydrated oocytes because of the presence of multiple developmental stages (Hunter and Macewicz, 1985). The presence of different vitellogenic stages in the ovary of a repeat-spawning fish makes it necessary to determine individual batch fecundity and spawning frequency before any estimate of annual fecundity can be made. In isochronal spawning fishes, such as striped mullet, this process is made simpler by the fact that oocytes mature at a similar rate (Greeley et al., 1987). During early vitellogenesis (180 μm to 350 μm), there is a higher degree of variability in the rate of development and a range of developmental stages would be present from the presence of precortical alveoli through secondary and tertiary vitellogenesis (Render et al., 1995). Estimating numbers of oocytes (uniform in an individual but varying in size because of season) during this stage would naturally make oocyte density and oocyte-size relationships inconsistent. This could possibly be corrected by using some timing factor such as month during the spawning season. The present study did demonstrate an inverse relationship between oocyte density and oocyte diameter. When month of capture was taken into consideration, oocyte density decreased with increasing oocyte size as the spawning season progressed.

In conclusion there were several biological aspects of striped mullet reproduction demonstrated in this study. Fecundity levels in striped mullet increased with total

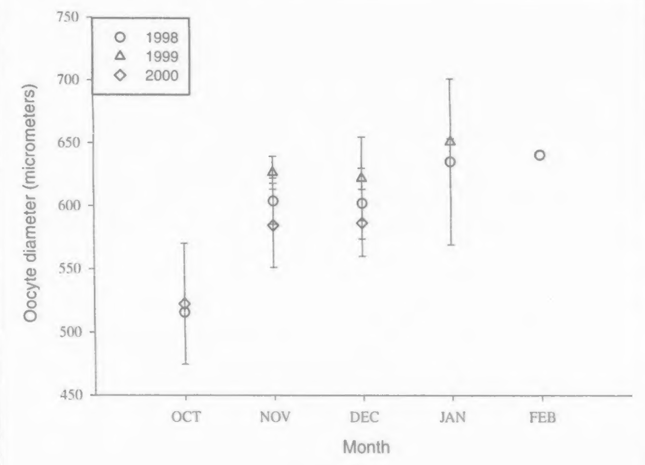


Figure 10

Mean oocyte diameter per month during the spawning season for striped mullet in South Carolina estuaries 1998 to 2000. Error bars represent standard deviation.

length (TL) and body weight (*BW=TW–OW*). Oocyte density remained relatively stable with size in the fecund fish and this allowed reasonable estimates of potential fecundity based on total length and body weight. Age-specific fecundity was highly variable and there appeared to be no consistent relationship. The reproductive season for striped mullet in South Carolina extends from October through April as determined by mean monthly GSI levels and histological confirmation of reproductive state. Potential spawning periods would also occur within this period as evidenced by the elevated GSI levels and the presence of atretic (or possible postspawning) ovaries from December through May. The gonadosomatic index itself is more useful to evaluate reproductive potential when used in conjunction with other techniques, such as histological analysis and oocyte diameter. The models of potential fecundity as they relate to size (total length and body weight) could be useful when applied to catch statistics of length and weight in populations with known size- and age- frequency distributions. This application would allow reasonable estimates of potential fecundity for these populations.

Acknowledgments

This study would not have been possible without the assistance of everyone in the Inshore Fisheries group at the Marine Resources Research Institute of the South Carolina Department of Natural Resources, which includes Myra Brouwer, John Archambault, Hayne Von Kolnitz, Will Hegler, Erin Levesque, Alice Palmer, Chad Johnson, Richie Evitt, Larry Goss, and Travis Waits. We especially thank Chad Altman of the South Carolina Department of Health and Environmental Control for the freshwater specimens. We also thank Myra Brouwer for assistance with map figures and the anonymous referees for careful review and suggestions for this manuscript. This research was made possible by National Marine Fisheries Service MARFIN, grant no. NA77FF0550.

Literature citations

Anderson, W. W.
1958. Larval development, growth, and spawning of striped mullet (*Mugil cephalus*) along the south Atlantic coast of the United States. Fish. Bull. 58:501–519.

Alvarez-LaJonchere, L.
1982. The fecundity of mullet (Pisces, Mugilidae) from Cuban waters. J. Fish Biol. 21:607–613.

Arnold, E. L., and J. R. Thompson.
1958. Offshore spawning of the striped mullet, *Mugil cephalus*, in the Gulf of Mexico. Copeia 1958:130–132.

Broadhead, G. C.
1956. Growth of the black mullet, *Mugil cephalus*, in west and northwest Florida. Mar. Lab. Tech. Ser. 25:1–29.

Campana, S. E., M. C. Annand, and J. I. McMillan.
1995. Graphical and statistical methods for determining the consistency of age determinations. Trans. Am. Fish. Soc. 124:131–138.

Chubb, C. F., I. C. Potter, C. J. Grant, R. C. J. Lenanton, and J. Wallace.
1981. Age, structure, growth rates, and movements of sea mullet, *Mugil cephalus* L., and yellow eye mullet, *Aldrichetta forsteri* (Valenciennes), in the Swan-Avon river system, Western Austrailia. Aust. J. Mar. Freshw. Res. 32:605–628.

Collins, M. R., and B. W. Stender.
1989. Larval striped mullet (*Mugil cephalus*) and white mullet (*Mugil curema*) off the southeastern United States. Bull. Mar. Sci. 45(3):580–589.

DeMartini, E. E., and B. B. Lau.
1999. Morphometric criteria for estimating sexual maturity in two snappers, *Etelis carbunculus* and *Pristipomoides seiboldii*. Fish. Bull. 97:449–458.

De Vlaming, V. L., H. S. Wiley, G. Delahunty, and R. Wallace.
1980. Goldfish (*Carassius auratus*) vitellogenin: induction, isolation, properties and relationship to yolk proteins. Comp. Biochem. Physiol. B 67B:613–623.

Dindo, J. J., and R. MacGregor.
1981. Annual cycle of serum gonadal steroids and serum lipids in striped mullet. Trans. Am. Fish. Soc. 110:403–409.

Finucane, J. H., L. A. Collins, and L. E. Barger.
1978. Spawning of the striped mullet, *Mugil cephalus*, in the northwestern Gulf of Mexico. Northeast Gulf Sci. 2: 148–150.

Fox, M. G., and A. J. Crivelli.
1998. Body size and reproductive allocation in a multiple spawning centrarchid. Can. J. Fish. Aquat. Sci. 55(3): 737–748.

Greeley, M. S., D. R. Calder, and R. A. Wallace.
1987. Oocyte growth and development in the striped mullet, *Mugil cephalus*, during sesonal ovarian recrudescence: relationship to fecundity and size at maturity. Fish. Bull. 85: 187–200.

Hettler, W. F., D. S. Peters, D. R. Colby, and E. H. Laban.
1997. Daily variability in abundance of larval fishes inside Beaufort Inlet. Fish. Bull. 95:477–493.

Humason, G. L.
1967. Animal tissue techniques, 426 p. W.H. Freeman and Co., San Francisco, CA.

Hunter, J. R., and B. J. Macewicz.
1985. Measurement of spawning frequency in multiple spawning fishes. *In* An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy, *Engraulis mordax*. NOAA Tech. Rep. NMFS 36:79–94.

Jacot, A. P.
1920. Age, growth, and scale characters of the mullets, *Mugil cephalus* and *Mugil curema*. Trans. Am. Fish. Soc. 39(3): 199–229.

Kaunda-Arara, B., and M. J. Ntiba.
1997. The reproductive biology of *Lutjanus fulviflamma* (Forsskål, 1775) (Pisces: Lutjanidae) in Kenyan inshore marine waters. Hydrobiologia 353(1/3):153–160.

Kelly, C. D.
1990. Effects of photoperiod and temperature on ovarian maturation in the striped mullet, *Mugil cephalus*. Pac. Sci. 44(2):187–188

Korhola, A., P. Solemdal, P. Bratland, and M. Fonn.
1996. Variation in annual egg production in individual captive Atlantic cod (*Gadhus morhua*). Can. J. Fish. Aquat. Sci. 53(3):610–620.

Kuo, C. M.
1995. Manipulation of ovarian development and spawning in grey mullet, *Mugil cephalus* L. Israel J. Aquacult. Bamidgeh 47(2):43–58.

Kuo, C. M., C. E. Nash, and Z. H. Shehadeh.
1974. A procedural guide to induce spawning in grey mullet (*Mugil cephalus* L.). Aquaculture 3(1974):1–14.

McDonough, C. J., and C. A. Wenner.
2003. Growth, recruitment, and abundance of juvenile *Mugil cephalus* in South Carolina estuaries. Fish. Bull. 101:343–357.

Okumus, I., and N. Bascinar.
1997. Population structure, growth, and reproduction of introduced mullet, *Mugil so-iuy*, in the Black Sea. Fish. Res. 33:131–137.

Pien, P. C., and J. C. Liao.
1975. Preliminary report of histological studies on the grey mullet gonad related to hormone treatment. Aquaculture 5:31–39.

Render, J. H., B. A. Thompson, and R. L. Allen.
1995. Reproductive development of striped mullet in Louisiana estuarine waters with notes on the applicability of reproductive assessment methods for isochronal species. Trans. Am. Fish. Soc. 124(1):26–36.

Rossi, A. R., M. Capula, D. Crosetti, D. E. Campton, and L. Sola.
1998. Genetic divergence and phylogenetic inferences in five species of Mugilidae (Pisces: Perciformes). Mar. Biol. 131: 213–218.

Shehadeh, Z. H., C. M. Kuo, and K. K. Milisen.
1973a. Validation of an *in vivo* method for monitoring ovarian development in the grey mullet (*Mugil cephalus* L.). J. Fish Biol. 1973(5):489–496.

Shehadeh, Z. H., W. D. Madden, and T. P. Dohl.
1973b. The effect of exogenous hormone treatment on spermiation and vitellogenesis in the grey mullet, *Mugil cephalus* L. J. Fish Biol. 1973(5):479–487.

Shireman, J. V.
1975. Gonadal development of striped mullet (*Mugil cephalus*) in freshwater. Prog. Fish Cult. 37(4):205–208.

Stenger, A. H.
1959. A study of the structure and development of certain reproductive tissues of *Mugil cephalus* Linnaeus. Zoologica 44(2):53–70.

Tamaru, C. S., C. S. Lee, C. D. Kelley, G. Miyamoto, and A. Moriwake.
1994. Oocyte growth in the Striped mullet, *Mugil cephalus* L., at different salinities. J. World Aquacult. Soc. 25: 109–115.

Thomson, J. M.
1951. Growth and habits of the sea mullet, *Mugil dobula* Gunther, in Western Australia. Aust. J. Mar. Freshw. Res. 2:193–225.
1963. Mullet life history strategies. Aust. J. Sci. 25:414–416. The grey mullets. Oceanogr. Mar. Biol. Annu. Rev. 4: 301–335.

Wallace, R. A., and K. Selman.
1981. Cellular and dynamic aspects of oocyte growth in teleosts. Am. Zool. 21:325–343.

Wenner, C. A., B. A. Roumillat, and C. W. Waltz.
1986. Contributions to the life history of black seabass, *Centropristis striata*, off the southeastern United States. Fish. Bull. 84:723–741.

Zar, J. H.
1984. Biostatistical analysis, 2nd ed., p. 292–305. Prentice Hall Inc., Englewood Cliffs, NJ.

Marine fish assemblages associated with fish aggregating devices (FADs): effects of fish removal, FAD size, fouling communities, and prior recruits

Peter A. Nelson

Department of Biological Sciences
Northern Arizona University
Flagstaff, Arizona 86011-5640
Present address: Center for Marine and Biodiversity & Conservation
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, California 92093-0202
E-mail address: pnelson@ucsd.edu

Abstract—Fishes are widely known to aggregate around floating objects, including flotsam and fish aggregating devices (FADs). The numbers and diversity of juvenile fishes that associated with floating objects in the nearshore waters of the eastern tropical Pacific were recording by using FADs as an experimental tool. The effects of fish removal, FAD size, and the presence or absence of a fouling community at the FAD over a period of days, and the presence of prior recruits over a period of hours were evaluated by using a series of experiments. The removal of FAD-associated fish assemblages had a significant effect on the number of the dominant species (*Abudefduf troschelii*) in the following day's assemblage compared to FADs where the previous day's assemblage was undisturbed; there was no experimental effect on combined species totals. Fishes do, however, discriminate among floating objects, forming larger, more species-rich assemblages around large FADs compared to small ones. Fishes also formed larger assemblages around FADs possessing a fouling biota versus FADs without a fouling biota, although this effect was also closely tied to temporal factors. FADs enriched with fish accumulated additional recruits more quickly than FADs that were not enriched with fish and therefore the presence of prior recruits had a strong, positive effect on subsequent recruitment. These results suggest that fish recruitment to floating objects is deliberate rather than haphazard or accidental and they support the hypothesis that flotsam plays a role in the interrelationship between environment and some juvenile fishes. These results are relevant to the use of FADs for fisheries, but emphasize that further research is necessary for applied interests.

Manuscript approved for publication
13 June 2003 by Scientific Editor.
Manuscript received 26 June 2003
at NMFS Scientific Publications Office.
Fish. Bull. 101:835–850

Fishes associate with floating objects in nearly all oceans of the world (Gooding and Magnuson, 1967; Hunter and Mitchell, 1967; Klima and Wickham, 1971; Crawford and Jorgenson, 1993; Kingsford, 1993; Druce and Kingsford, 1995; Massuti et al., 1998; Hampton and Bailey, 1999; Parin and Fedoryako, 1999). Fishes also gather around fish aggregating devices (FADs), floating objects deployed to concentrate target species or bait fishes and improve the catch for artisanal, sport, or commercial fisheries. The physical attributes of a floating object, such as a FAD, may affect the ability of potential fish recruits to locate the floating object or may affect the adaptive advantages of associating with that object (or both)—a topic that has been addressed in numerous prior studies (e.g. Hunter and Mitchell, 1968; Wickham et al., 1973; Wickham and Russell, 1974; Fedoryako, 1989; Rountree, 1989; Safran, 1990; Safran and Omori, 1990; Friedlander et al., 1994; Hall et al., 1999b). However, the present study is apparently the first to address empirically the effects of disturbance, fouling communities, and prior recruits by examining both the number and the diversity of fishes that aggregate around FADs. In addition, this study addresses the effect of FAD size, a factor well represented in prior studies but frequently confounded by temporal or design issues. FADs are widely used to enhance sport and commercial fisheries, but are expensive to build, deploy, and maintain; therefore better information about the effects of FAD size and fouling could aid design efforts. Given the bycatch associated with the FAD fishery for tuna in the eastern tropical Pacific (Hall et al., 2000), for example, we need a better understanding of how fishes use FADs in order to manage fisheries for FAD-associated species (Lennert-Cody and Hall, 2000). Finally, careful study of how differing characteristics of floating objects affect fish recruitment may provide important clues regarding the adaptive significance of fish associations with flotsam and drift algae—a phenomenon widely noted but poorly understood.

Prior research has suggested that rates of immigration and fish removal from FADs similar to those seen in the present study were high from one day to the next (Nelson, 1999), and Wickham and Russell (1974) reported that mid-water FADs, which were fished daily, produced a larger cumulative catch than mid-water FADs, which were undisturbed during the same period and then fished once at the end of the conclusion of the study. I tested the hypothesis that, over time, the size and diversity of FAD-associated fish assemblages are reduced by the repeated removal of these fishes compared with undisturbed assemblages. Effective management or use of FADs deployed for fisheries purposes and an understanding of the ecological relationship between flotsam and fishes associated with flotsam will depend in part on patterns of immigration and loss to fish assemblages.

There have been numerous attempts to equate flotsam structure (size, complexity, orientation, etc.) with the number of associated fishes (e.g. Hunter and Mitchell, 1968; Dooley, 1972; Wickham et al., 1973; Wickham and Russell, 1974; Rountree, 1989; Druce and Kingsford, 1995), but the results have been equivocal, except when the analysis was restricted to a single species (e.g. *Histrio histrio*, Dooley, 1972; *Decapterus punctatus*, Rountree, 1989). Huge aggregations have been associated with very small objects—IATTC (Inter-American Tropical Tuna Commission) records include a report of 55 metric tons of mostly yellowfin tuna (*Thunnus albacares*) fished from beneath a 1-m length of floating polypropylene rope (Hall et al., 1999b); therefore, despite the intuitive appeal, there is no clear reason to expect that size of FAD *per se* is an important factor in determining the size of associated assemblages. Thus, object size remains an unresolved problem in understanding flotsam-associated communities. If there are optimal FAD sizes, these may be species specific, and economical FAD design depends upon controlled experiments in the field.

Fouling organisms (sessile invertebrates and algae that colonize flotsam) are believed to have a strong, positive effect on the subsequent recruitment and retention of fishes by commercial fishermen (Gaertmer and Medina-Gaertner, 1999; Hall et al., 1999a; Hallier and Parajua, 1999; Suzuki, 1999). However, prior to the results presented here, there appear to have been no controlled tests of the hypothesis that the presence of fouling organisms enhances fish recruitment to a floating object. I also compared the numbers and diversity of fishes associated with FADs that are equipped with artificial (lead weight) fish versus FADs without these artificial fish. The latter experiment was intended to determine the importance of prior recruits to subsequent patterns of recruitment. To test a similar hypothesis over the short term (hours versus days) and using living fish instead of painted models, I also compared recruitment to FADs enriched with real fish (juvenile *Abudefduf troschelii*) to unenriched FADs.

I tested the hypothesis that each of these factors would affect the number of fishes associated with FADs (combined and individual species), as well as the species diversity of FAD-associated fish assemblages. Both the size of these FAD-associated fish assemblages and their species diversity provide insight on recruitment processes and the use of floating objects by fishes. Although the association of fishes with floating objects has been well documented, very little is known regarding the behavioral and ecological processes behind these assemblages. The results reported in the present study provide new information on the role of flotsam and FAD characteristics in determining the number and diversity of these assemblages, and some clues towards understanding why and how fishes aggregate beneath floating objects.

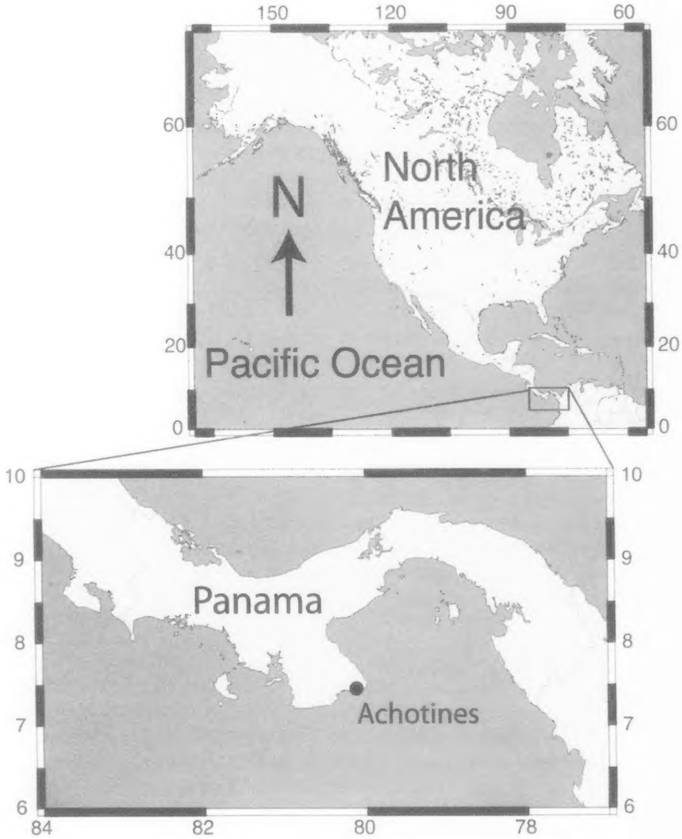


Figure 1

Location of the study site at Achotines, Panama, Central America.

Materials and methods

Study site and FAD construction

All research was conducted between July and October 1997 on the Pacific coast of Panama, Central America, from the Inter-American Tropical Tuna Commission laboratory at Achotines, near the tip of the Azuero Peninsula (Fig. 1). Experimental FADs were constructed of three tuna purse-seine buoys lashed together and anchored to the substrate with a 25-kg cast concrete block unless otherwise noted (Fig. 2). The length of the anchor lines allowed the FADs to rest at the surface at all tidal heights. Each buoy was roughly 25 cm in cross sectional diameter, and approximately 35 cm in length. The FADs were detachable from their moorings by detaching a large (2 m diameter) loop on the anchor line that held a 2-kg line weight (Fig. 2). This design allowed me to change FADs for another treatment. The FAD arrays were deployed nearshore (within 1.5 km;

Fig. 3) and in shallow water (14–25 m). The FAD treatments were not assigned randomly to FAD positions; instead, I assigned treatments uniformly across the FAD array because the total number of FADs was relatively low (8–10) and, given the small number of experimental units, in order to reduce the possibility that the results be confounded by positional effects.

The anchored FADs were spaced approximately 100 m apart. The maximum horizontal underwater visibility measured was 27 m, and typically averaged much less. Assuming that vision was the principal means by which fishes located these objects, it is therefore highly unlikely that fishes treated the FAD array as a single "object" or moved from FAD to FAD within the array. I believe that it was unlikely that any fish transferred from one FAD to another for the following reasons. 1) The horizontal underwater visibility was always much less than the distance between FADs. 2) Observations suggest that short-term, daytime fidelity was high; once a fish associated with a floating object, it was unlikely to leave that object during the day (Nelson, 1999). 3) Crossing an open stretch of water for another floating object (presumably within detection range) entails a potential risk for a fish. Moser et al. (1998) did note that the larger juveniles and adult fishes associated with floating *Sargassum* showed little apparent fidelity to this habitat and would move between their research boats, floating observation equipment, and the *Sargassum* habitat. However, the fishes that were observed to move between these floating objects were juvenile carangids between 10 and 20 cm in length (Moser et al., 1998), whereas the fishes in the present study were generally much smaller and presumably less vagile.

Longshore currents ran roughly west to east through the experimental area, and rarely in the reverse (Fig. 3). I recorded an estimate of current direction using an underwater compass and the angle of the FAD anchor lines. This estimate represented the sum of the forces due to windage on the FAD buoys and currents.

Censusing FADS

FAD-associated fish assemblages were censused by direct visual observation by divers using mask and snorkel. Most other studies of fish assemblages associated with floating material have employed nets or quantitative fishing methods for sampling purposes (e.g. Kojima, 1960; Dooley, 1972; Kingsford, 1992, 1995), but Hunter and Mitchell (1968) compared data from net captures, automated photography and direct visual observations and found that the visual observations agreed well with the other methods, and provided behavioral information not available with the other methods. In my study, a FAD was approached by swimming

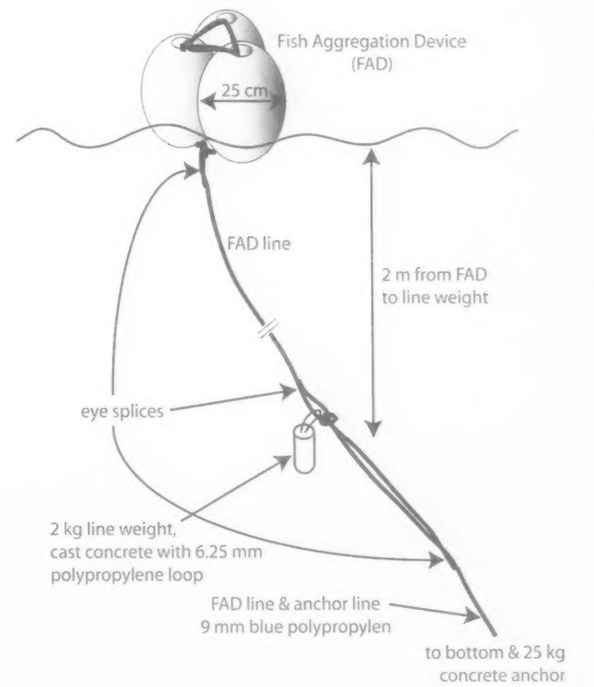

Fish Aggregation Device
(FAD)
25 cm
FAD line
2 m from FAD
to line weight
eye splices
2 kg line weight,
cast concrete with 6.25 mm
polypropylene loop
FAD line & anchor line
9 mm blue polypropylen
to bottom & 25 kg
concrete anchor

Figure 2

Design of detachable fish aggregating devices (FADs) used in the present study.

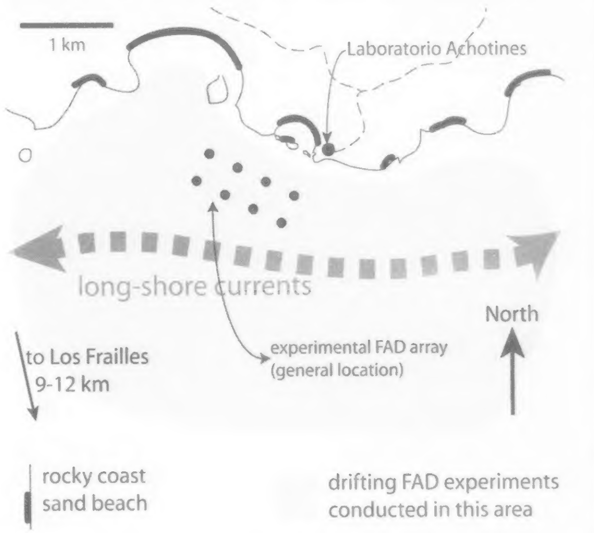

1 km
Laboratorio Achotines
long-shore currents
North
to Los Frailles
9-12 km
experimental FAD array
(general location)
rocky coast
sand beach
drifting FAD experiments
conducted in this area

Figure 3

Coastline and general location of experiments and observations. The laboratory (Laboratorio Achotines) is located at approximately 7°5′N lat, 80°10′W long.

There have been numerous attempts to equate flotsam structure (size, complexity, orientation, etc.) with the number of associated fishes (e.g. Hunter and Mitchell, 1968; Dooley, 1972; Wickham et al., 1973; Wickham and Russell, 1974; Rountree, 1989; Druce and Kingsford, 1995), but the results have been equivocal, except when the analysis was restricted to a single species (e.g. *Histrio histrio*, Dooley, 1972; *Decapterus punctatus*, Rountree, 1989). Huge aggregations have been associated with very small objects—IATTC (Inter-American Tropical Tuna Commission) records include a report of 55 metric tons of mostly yellowfin tuna (*Thunnus albacares*) fished from beneath a 1-m length of floating polypropylene rope (Hall et al., 1999b); therefore, despite the intuitive appeal, there is no clear reason to expect that size of FAD *per se* is an important factor in determining the size of associated assemblages. Thus, object size remains an unresolved problem in understanding flotsam-associated communities. If there are optimal FAD sizes, these may be species specific, and economical FAD design depends upon controlled experiments in the field.

Fouling organisms (sessile invertebrates and algae that colonize flotsam) are believed to have a strong, positive effect on the subsequent recruitment and retention of fishes by commercial fishermen (Gaertmer and Medina-Gaertner, 1999; Hall et al., 1999a; Hallier and Parajua, 1999; Suzuki, 1999). However, prior to the results presented here, there appear to have been no controlled tests of the hypothesis that the presence of fouling organisms enhances fish recruitment to a floating object. I also compared the numbers and diversity of fishes associated with FADs that are equipped with artificial (lead weight) fish versus FADs without these artificial fish. The latter experiment was intended to determine the importance of prior recruits to subsequent patterns of recruitment. To test a similar hypothesis over the short term (hours versus days) and using living fish instead of painted models, I also compared recruitment to FADs enriched with real fish (juvenile *Abudefduf troschelii*) to unenriched FADs.

I tested the hypothesis that each of these factors would affect the number of fishes associated with FADs (combined and individual species), as well as the species diversity of FAD-associated fish assemblages. Both the size of these FAD-associated fish assemblages and their species diversity provide insight on recruitment processes and the use of floating objects by fishes. Although the association of fishes with floating objects has been well documented, very little is known regarding the behavioral and ecological processes behind these assemblages. The results reported in the present study provide new information on the role of flotsam and FAD characteristics in determining the number and diversity of these assemblages, and some clues towards understanding why and how fishes aggregate beneath floating objects.

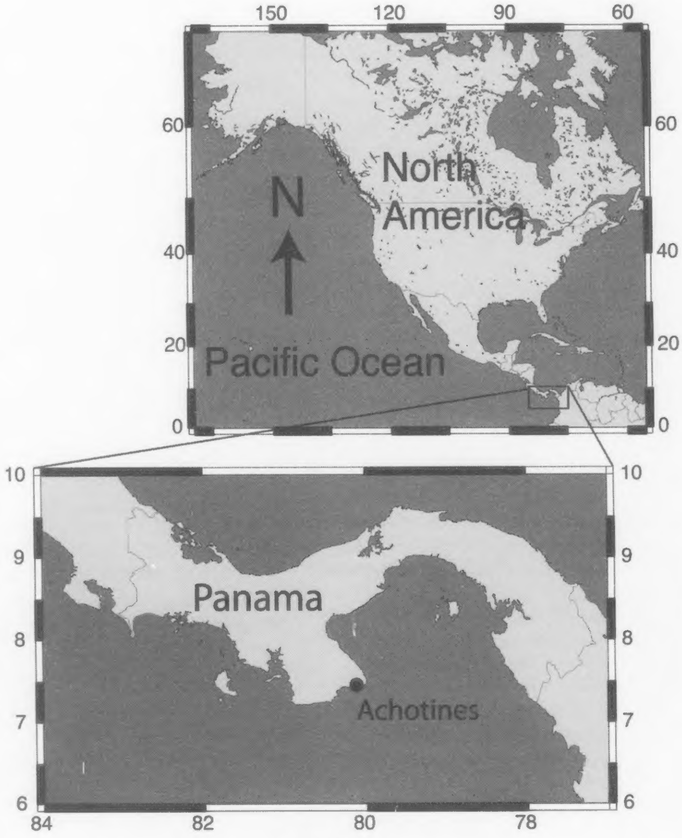


Figure 1

Location of the study site at Achotines, Panama, Central America.

Materials and methods

Study site and FAD construction

All research was conducted between July and October 1997 on the Pacific coast of Panama, Central America, from the Inter-American Tropical Tuna Commission laboratory at Achotines, near the tip of the Azuero Peninsula (Fig. 1). Experimental FADs were constructed of three tuna purse-seine buoys lashed together and anchored to the substrate with a 25-kg cast concrete block unless otherwise noted (Fig. 2). The length of the anchor lines allowed the FADs to rest at the surface at all tidal heights. Each buoy was roughly 25 cm in cross sectional diameter, and approximately 35 cm in length. The FADs were detachable from their moorings by detaching a large (2 m diameter) loop on the anchor line that held a 2-kg line weight (Fig. 2). This design allowed me to change FADs for another treatment. The FAD arrays were deployed nearshore (within 1.5 km;

Fig. 3) and in shallow water (14–25 m). The FAD treatments were not assigned randomly to FAD positions; instead, I assigned treatments uniformly across the FAD array because the total number of FADs was relatively low (8–10) and, given the small number of experimental units, in order to reduce the possibility that the results be confounded by positional effects.

The anchored FADs were spaced approximately 100 m apart. The maximum horizontal underwater visibility measured was 27 m, and typically averaged much less. Assuming that vision was the principal means by which fishes located these objects, it is therefore highly unlikely that fishes treated the FAD array as a single "object" or moved from FAD to FAD within the array. I believe that it was unlikely that any fish transferred from one FAD to another for the following reasons. 1) The horizontal underwater visibility was always much less than the distance between FADs. 2) Observations suggest that short-term, daytime fidelity was high; once a fish associated with a floating object, it was unlikely to leave that object during the day (Nelson, 1999). 3) Crossing an open stretch of water for another floating object (presumably within detection range) entails a potential risk for a fish. Moser et al. (1998) did note that the larger juveniles and adult fishes associated with floating *Sargassum* showed little apparent fidelity to this habitat and would move between their research boats, floating observation equipment, and the *Sargassum* habitat. However, the fishes that were observed to move between these floating objects were juvenile carangids between 10 and 20 cm in length (Moser et al., 1998), whereas the fishes in the present study were generally much smaller and presumably less vagile.

Longshore currents ran roughly west to east through the experimental area, and rarely in the reverse (Fig. 3). I recorded an estimate of current direction using an underwater compass and the angle of the FAD anchor lines. This estimate represented the sum of the forces due to windage on the FAD buoys and currents.

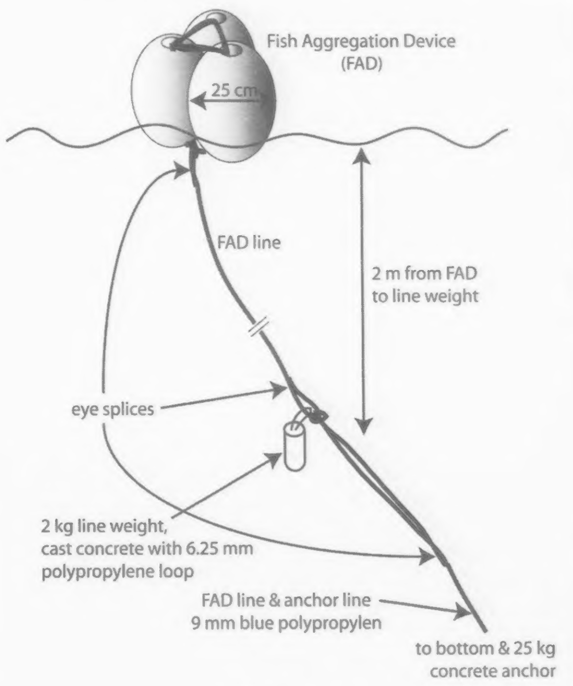

Fish Aggregation Device
(FAD)
25 cm
FAD line
2 m from FAD
to line weight
eye splices
2 kg line weight,
cast concrete with 6.25 mm
polypropylene loop
FAD line & anchor line
9 mm blue polypropylen
to bottom & 25 kg
concrete anchor

Figure 2

Design of detachable fish aggregating devices (FADs) used in the present study.

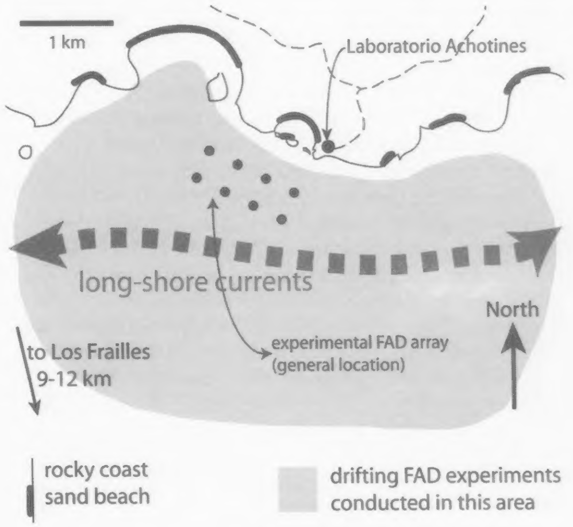

1 km
Laboratorio Achotines
long-shore currents
North
to Los Frailles
9-12 km
experimental FAD array
(general location)
rocky coast
sand beach
drifting FAD experiments
conducted in this area

Figure 3

Coastline and general location of experiments and observations. The laboratory (Laboratorio Achotines) is located at approximately 7°5′N lat, 80°10′W long.

Censusing FADS

FAD-associated fish assemblages were censused by direct visual observation by divers using mask and snorkel. Most other studies of fish assemblages associated with floating material have employed nets or quantitative fishing methods for sampling purposes (e.g. Kojima, 1960; Dooley, 1972; Kingsford, 1992, 1995), but Hunter and Mitchell (1968) compared data from net captures, automated photography and direct visual observations and found that the visual observations agreed well with the other methods, and provided behavioral information not available with the other methods. In my study, a FAD was approached by swimming slowly and quietly at the surface from a distance of at least 12 m. All fishes associated (defined below) with the floating object were counted and identified; therefore the statistical unit in all of the experiments described below was a single FAD-associated assemblage of fishes at a given date and time. Horizontal underwater visibility, measured with a Secchi disk, was always sufficient to allow the identification of species and to count individual fishes from a minimum distance of 2 meters.

Any fish observed within 2 m of a FAD was considered to be "FAD-associated." Very few fishes were observed outside of this range, and with rare exceptions, fishes responded to the approach of an observer first by swimming towards the observer and then by moving closer to the FAD, rather than away from it. Different species of fishes used the space around and below the FAD differently, as both Gooding and Magnuson (1967) and Hunter and Mitchell (1967) described, but the juvenile fishes that predominated in the present study were unambiguous regarding their relationship to the FADs. After fishes resumed their prior positions in relation to a FAD, continued observations of these fishes revealed that there was no inclination to abandon that FAD. The appearance of potential predators invariably resulted in a tightening of the spatial distribution around the FAD.

When the experiment required the capture of FAD-associated fishes, I used a smaller (1.1×1.3 m) version of the diver-operated liftnet described by McCleneghan and Houk (1978). Captured fishes were preserved for further studies, held in grow-out facilities in the laboratory to verify species identification, or released 1.5 km down-current over rocky reef habitat to ensure that they had effectively been removed from the FAD array.

Diversity calculations

Measurements of species diversity provided a means of monitoring treatment effects on the composition of FAD-associated assemblages. I measured species diversity using species richness (S, the raw number of species observed), and the Brillouin index (HB). S is simple and widely used, but increases with sample size, and, where sample sizes are unequal, HB provides a less biased measure of diversity (Magurran, 1988). In addition, HB was chosen over one of the more commonly used information theory indices (e.g. the Shannon-Wiener index) because 1) FAD-associated assemblages are not a random sample of potential recruits (different species vary in their attraction to floating objects) and 2) each of these assemblages was completely censused—not sampled (Magurran, 1988, and references therein).

HB is calculated as

$$HB = \frac{\ln N! - \sum \ln n_i!}{N},$$

where N = the total number of fishes of all species observed; and
n_i = the number of individuals within the ith species (Magurran, 1988).

Statistical analyses

I used a two-way repeated measures ANOVA (α=0.05) to test for treatment differences, differences among observation dates and evidence of treatment-by-sample interaction for assemblage sizes (no. of fish(es)), species richness (S) and species diversity (HB). Because individual species differed in their relative abundance and had different ecological requirements, there was the potential for the dominant species to bias comparisons of experimental treatments where assemblage size (a combinination of all species) was used. For all experiments except for the recruit-enriched experiment, I repeated the statistical analyses twice: once using the number of sergeant major damselfish (*Abudefduf troschelii*) only and again using all fishes combined but with *A. troschelii* removed. For the artificial fish experiment where rainbow runner (*Elagatis bipinnulata*) were particularly abundant, I ran separate analyses for *A. troschelii* alone, *E. bipinnulata* alone, and for all species minus the numbers of *A. troschelii*. When no fishes were present at a FAD, HB was undefined; the "missing" data were replaced according to the procedures of Zar (1996) and the degrees of freedom were reduced accordingly.

Fish-removal experiments

Observations on similar FADs during a previous field season at the same location suggested that the turnover rate of fish associated with anchored FADs is high, especially when the initial assemblage is large, but that some recognizable individuals did persist from day-to-day (Nelson, 1999). If immigration and emigration rates were as high as suspected, FADs cleared of fish on a daily basis should not differ significantly from undisturbed FADs in their mean assemblage size or in the average number of species associated with these FADs. Wickham and Russell (1974) compared the catches of bait fishes associated with FADs subject to daily purse-seine sets versus those allowed to "soak" undisturbed for three days prior to a single purse-seine set and concluded that sufficient emigration and immigration occurred on a daily basis to remove any appreciable effect of daily removals. I sought to address similar questions, but by using a different system (juvenile reef fishes versus bait fishes).

To test these hypotheses, I deployed eight identical FADs on 30 June 1997 in two lines of four FADs each, oriented roughly parallel to shore (Figs. 2 and 3). Fishes associated with all eight FADs were counted and identified on a daily basis, beginning 3 July 1997. Alternate FADs were cleared of all fish, following the daily counts; the remaining FADs were left undisturbed in such a way as to distribute the treatments evenly among the FAD array. After three consecutive days of these observations (series 1), the treatments were reversed, and previously undisturbed FADs were cleared, and those that had been cleared regularly were left undisturbed (series 2). The treatments were reversed in an attempt to control for possible positional effects of the FADs. However, the two series were necessarily run consecutively, not concurrently; therefore treatment position was confounded by sample date. I used a 2 (cleared vs.

undisturbed) by 2 (first series vs. second series) by 6 (sample date) model and I used a repeated measures ANOVA (repeated on sample date) on the following dependent variables: assemblage size (total no. of fishes), species richness (S), and HB. I repeated analyses of assemblage-size effects looking at the number of *A. troschelii* only, and the total number of fishes minus the number of *A. troschelii*.

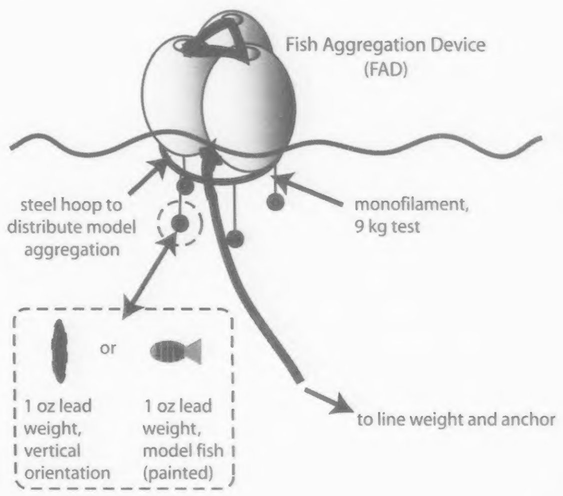

Fish Aggregation Device (FAD)
steel hoop to distribute model aggregation
monofilament, 9 kg test
or
1 oz lead weight, vertical orientation
1 oz lead weight, model fish (painted)
to line weight and anchor

Figure 4

Treatments for the artificial fish experiment involved suspending artificial fish, lead weights of an equivalent mass and volume, or nothing (control) from a steel hoop lashed beneath the FAD.

FAD size

To determine the effect of FAD size on the associated assemblage size and diversity, I compared FAD-associated fish assemblages between triple-size FADs and single FADs. An existing anchored array of eight FADs (two lines parallel to the coast of four FADs each, Fig. 3) was cleared of fishes on 24 July 1997. As the fish were removed from the FADs, each FAD was replaced with a fresh (i.e. clean and unfouled) single or triple-size FAD, placed at alternating positions. The single FADs were constructed as described above and in Figure 2; the triple-size FADs were identical to the single FADs, except that they consisted of nine, rather than three, purse-seine buoys lashed together and had the effect of nearly tripling the wetted surface area (although inner buoys are less exposed than outer ones) and the volume of the FAD, and of increasing the maximum linear dimension of the FAD by a factor of two. Treatments were not reversed for this or subsequent experiments because sample date appeared to be the major factor determining assemblage size for any species, based on the previous experiment. Note that in each of these experiments, except for the recruit-enrichment experiment that used drifting FADs, treatments were assigned uniformly throughout the FAD arrays so that onshore, offshore, or longshore biases in recruitment due to oceanographic processes would not confound the results. Fishes at all FADs were counted and identified on three dates (26, 28, and 30 July 1997), each observation separated from the next by 48 hours. No fish were collected, with the exception of one balistid, taken from the array on 26 July because it was the first of that species to be observed associated with a FAD. Data were analyzed for experimental effects on total assemblage size, species diversity (S and HB), the number of *A. troschelii,* and total number of fishes minus the number of *A. troschelii*.

Presence of absence of a fouling community

To determine whether the presence of a fouling community on a floating object affected the associated fish assemblage, I compared FAD-associated assemblage sizes and species richness between fouled and unfouled (control) FADs. Control FADs were scrubbed of all fouling organisms. Fouled FADs had been deployed for a minimum of 14 days (range: 14–22 days) in the study area, and had accumulated fouling that completely covered the wetted surface of the FAD with gooseneck barnacles (*Lepas* sp.), hydroids, and bryozoans. Grapsid crabs and polychaete worms (*Amphimone vagans*) were also intermittent associates of fouled FADs. Control and fouled FADs were deployed on 8 September 1997 in an alternating array of eight buoys, with four FADs per treatment (layout and spatial distribution of treatments follow that of the FAD size experiment). All fishes were cleared from FAD positions prior to deploying the FADs, and data collection commenced 24 hours later. Data were collected on four consecutive days (9, 10, 11, and 12 September 1997) and analyzed for experimental effects on total assemblage size, species diversity (S and HB), the number of *A. troschelii*, the number of *E. bipinnulata*, and total number of fishes minus the number of *A. troschelii*.

Artificial fish experiment

I tested the hypothesis that potential recruits would distinguish between FADs with an "assemblage" of artificial fish suspended beneath them, FADs with an "assemblage" of suspended material equal to the artificial fish in size but not resembling fish in appearance, and control FADs without anything suspended beneath them (Fig. 4). I constructed artificial fish from 31.25-g lead fishing weights. These weights were flattened, tear-drop–shaped objects, painted a dull yellow with black bars to resemble juvenile *Abudefduf troschelii* and suspended, by using monofilament (20 lb. test) and a steel hoop, beneath the "artificial fish FADs" (Fig. 4). I suspended oblong 31.25-g lead fishing weights beneath "weighted FADs," and the control FADs had only a steel hoop beneath each (Fig. 4). These FADs were deployed in an anchored array, and the various treatments were distributed in an alternating pattern throughout the array. FAD positions were cleared of fishes,

Table 1

Fish species and life history stages observed at all experimental FADs (combined data) with relative importance by frequency and abundance. 1 = coastal pelagic species; 2 = substrate-associated species; and 3 = possible flotsam specialists. J = juvenile; A = adult.

No.	Species	Family	Stage	Frequency (%)	Abundance (%)
1	*Abudefduf troschelii*[2]	Pomacentridae	J	34.1	81.4
2	*Elagatis bipinnulata*[1]	Carangidae	J	16.9	8.1
3	*Polydactylus approximans*[2]	Polynemidae	J	6.4	1.8
4	*Mugil* sp.[2]	Mugilidae	J	6.4	1.3
5	*Lutjanus argentiventris*[2]	Lutjanidae	J	6.4	1.1
6	*Epinephelus panamensis*[2]	Serranidae	J	3.4	0.5
7	*Hoplopagrus guntheri*[2]	Lutjanidae	J	3.2	0.5
8	*Canthidermis maculatus*[1]	Balistidae	J	2.8	0.4
9	*Gnathanodon speciosus*[2]	Carangidae	J	1.5	0.2
10	*Alectis ciliaris*[1]	Carangidae	J		
11	*Caranx caninus*[1]	Carangidae	J		
12	*Caranx caballus*[1]	Carangidae	J	14.7	3.9
13	*Caranx vinctus*[1]	Carangidae	J	(nos. 10–14)	
14	*Seriola peruana*[1]	Carangidae	J		
15	*Tylosaurus acus pacificus*[1]	Belonidae	A		
16	*T. crocodilus fodiator*[1]	Belonidae	A		
17	*Fistularia commersonii*[2]	Fistulariidae	J		
18	*Syngnathus auliscus*[2]	Syngnathidae	J		
19	*Lobotes pacificus*[3]	Lobotidae	J and A		
20	*Mulloidichthys dentatus*[2]	Mullidae	J	4.1	0.7
21	*Sectator ocyurus*[3]	Kyphosidae	A	(nos. 15–26)	
22	*Parapsettus panamensis*[2]	Ephippidae	J		
23	*Hypsoblennius breviceps*[2]	Blenniidae	?		
24	goby (unidentified)[2]	Gobiidae	?		
25	*Aluterus scriptus*[2, 3]	Balistidae	J and A		
26	*Balistes polylepis*[2]	Balistidae	J		
	26 species	16 families		100	100

and treatment FADs were deployed on 24 September 1997. FADs were monitored daily as described above, from 25 September through 3 October 1997 (sampling days=9). Data were analyzed for experimental effects on total assemblage size, species diversity (S and HB), number of *A. troschelii*, number of *E. bipinnulata*, and total number of fishes minus the number of *A. troschelii*.

Recruit-enriched vs. nonenriched FADs

I tested the hypothesis that the presence of prior recruits (juvenile sergeant major damselfish, *Abudefduf troschelii*) would have a positive effect on subsequent recruitment to a FAD. I used *A. troschelii* because these were the most important species associated with FADs by frequency and abundance (Table 1). It is possible that the selection of a particular species as the prior recruit might affect the subsequent recruitment of the same or different species (via intra- or interspecific competition for example), but I had no basis for predicting the direction of such effects.

Given the strong day-to-day changes in assemblage sizes, this test required frequent, short-interval observations of the experimental FADs. I used drifting, rather than anchored, FADs to provide a more realistic (and conservative) test of the effect. (Drifting objects should result in fewer chance encounters by potential fish recruits carried by currents through a fixed FAD array, but anchored FADs are much easier to track for longer experiments.) I deployed four drifting FADs (constructed from 3 buoys—the "single" size) in the stippled area indicated in Figure 3. Two of these FADs were enriched with nine *A. troschelii* per FAD, previously collected from anchored FADs and released in close proximity to drifting FADs immediately after deployment. The two control FADs received no sergeant majors to start. Both groups were checked immediately following deployment to verify that the fish had associated with the experimental FADs and to check against quick recruitment to the control FADs. To minimize the potential transfer of fish with the boat, I accelerated sharply when leaving a FAD enriched with sergeant majors and when checking the FADs, entered the water from the boat a minimum of 10 m from each FAD.

The FADs were deployed from an inflatable boat at 50-m intervals in a roughly linear array, and checked at hourly intervals. The FADs did not maintain their initial spatial arrangement, but I did not move any FAD once the drift began unless FAD-to-FAD distance had been reduced to less than 10 m. In this instance, I moved one or more FADs to a minimum FAD-to-FAD distance of 50 m after checking for any FAD-associated fishes. In none of these instances were any FAD-associated fishes observed. I monitored the drift for four hours; deteriorating weather and fading light, however, did not permit additional observations.

I used linear regression to test the hypothesis that the number of FAD-associated fishes changed over time for the enriched FADs and for the nonenriched FADs. I used a t-test to compare the slopes of the two regression models and to test the hypothesis that the treatments accumulated fish at different rates.

Results

Twenty-six species of fishes from 16 families were recorded, including species associated with reef, soft bottom, and coastal pelagic habitats as adults (Table 1). Only juvenile specimens were observed clearly associated with FADs, with the exception of *Aluterus scriptus* and *Lobotes pacificus*, of which both juvenile and adult forms were observed in close, continuous proximity to the FADs. Two needlefish species (*Tylosaurus acus pacificus* and *T. crocodilus fodiator*) appeared occasionally in close proximity to the FADs, but they were not clearly associated with the FADs. An adult *Lobotes pacificus* (tripletail) was observed once and a single adult *Aluterus scriptus* (scrawled filefish) were observed on three separate instances. Horizontal underwater visibility averaged 13.4 m (±1.7 SE) for all sampling days combined.

Juvenile sergeant major damselfish (*Abudefduf troschelii*) were the dominant species by frequency of occurrence and numerical abundance (Table 1) for all experiments. The damselfish was followed in rank overall by juvenile rainbow runner (*Elagatis bipinnulata*), although this species was observed with the FADs only during the fouling and model fish experiments. Juvenile threadfin (*Polydactylus approximans*), mullet (*Mugil* sp.), and yellow snapper (*Lutjanus argentiventris*) were equally frequent but differed slightly in abundance (*P. approximans*>*Mugil* sp.>*L. argentiventris*; Table 1). The latter pattern was consistent across all experiments. Specimens from a suite of juvenile carangids (excluding *E. bipinnulata*) were also observed frequently.

Fish-removal experiments

Sample date, series, and treatment combined to have a significant effect on *A. troschelii* abundance (three way interaction, P=0.03), but there was no clear pattern; the remaining species (combined species less numbers of *A. troschelii*) were influenced by sample date (date by series interaction, P<0.01) but not by treatment (P=0.73, Table 2, Fig. 5). Measures of diversity varied between series (series: S, P<0.01; HB, P=0.01) but were unaffected by treatment. Thus, fish removal or fish disturbance may contribute to assemblage sizes for individual species (e.g. *A. troschelii*), but, in the present study, the total number of combined species was unaffected.

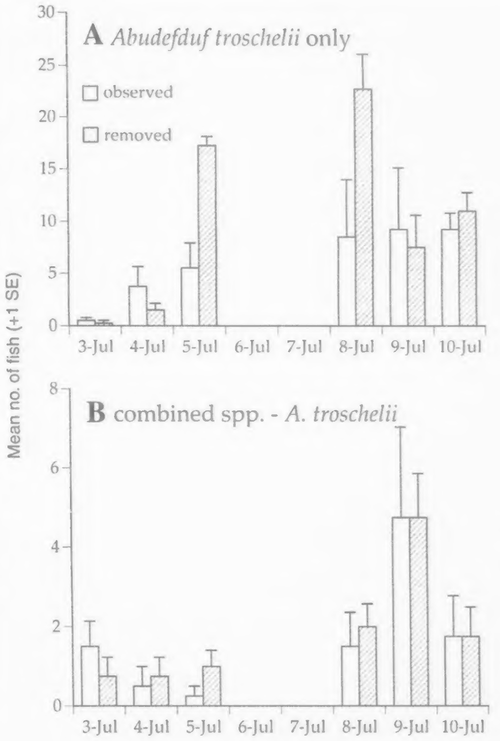


Figure 5

Repeated fish-removal effects (disturbed vs. undisturbed [observed only]) on aggregation size (mean no. of fishes) for *Abudefduf troschelii* alone (**A**) and for all species combined less *A. troschelii* (**B**). See Tables 3–5 for sample sizes and ANOVA results for assemblage size and diversity measures.

FAD size

Abudefduf troschelii was strongly affected by a combination of treatment and sample date (date by treatment interaction, P=0.03, Table 3, Fig. 6). Results from the remaining species combined were comparable with larger total numbers at the larger FADs, although not statistically significant (treatment, P=0.07). Although both measures of diversity (S and HB) suggested that the treatment may have had a positive effect on diversity (S, treatment, P=0.02), species richness was positively correlated with sample size. HB, a diversity measure comparatively unaf-

Table 2

Repeated measures ANOVA results (cleared FADs vs. undisturbed FADs, $n_1=n_2=12$) in fish-removal experiment.

Dependent variable	Factor(s)	F	df	P	$1-\beta$
Number of fishes (all species combined)	treatment	0.12	1, 12	0.73	0.06
	series	35.3	1, 12	<0.01	>0.99
	treatment × series	0.64	1, 12	0.44	0.11
	date	5.53	2, 24	0.01	0.81
	date × series	48.5	2, 24	<0.01	>0.99
	date × treatment	3.22	2, 24	0.06	0.55
	3-way interaction	0.06	2, 24	0.94	0.06
Number of fish (*A. troschelii* only)	treatment	16.7	1, 12	<0.01	0.97
	series	5.92	1, 12	0.03	0.61
	treatment × series	0.27	1, 12	0.61	0.08
	date	3.10	2, 24	0.06	0.53
	date × series	7.71	2, 24	<0.01	0.93
	date × treatment	2.95	2, 24	0.07	0.51
	3-way interaction	4.24	2, 24	0.03	0.69
Number of fishes (all spp. minus *A. troschelii*)	treatment	0.05	1, 12	0.82	0.06
	series	13.3	1, 12	<0.01	0.93
	treatment × series	0.01	1, 12	0.94	0.05
	date	2.93	2, 24	0.07	0.51
	date × series	4.06	2, 24	0.03	0.66
	date × treatment	0.07	2, 24	0.93	0.06
	3-way interaction	0.31	2, 24	0.74	0.09
Species richness (S)	treatment	0.63	1, 12	0.44	0.11
	series	11.8	1, 12	<0.01	0.90
	treatment × series	0.63	1, 12	0.44	0.11
	date	0.43	2, 24	0.66	0.11
	date × series	0.63	2, 24	0.54	0.14
	date × treatment	0.69	2, 24	0.51	0.15
	3-way interaction	2.08	2, 24	0.37	0.37
Species diversity (HB)[1]	treatment	1.05	1, 10	0.33	0.15
	series	8.79	1, 10	0.01	0.79
	treatment × series	0.54	1, 10	0.48	0.10
	date	1.43	2, 22	0.26	0.27
	date × series	2.80	2, 22	0.08	0.49
	date × treatment	0.12	2, 22	0.12	0.07
	3-way interaction	0.61	2, 22	0.55	0.14

[1] Missing data were replaced according to the directions in Zar (1996), and the degrees of freedom were reduced accordingly. "3-way interaction" refers to interactions between treatment, series, and dates.

fected by sample size (Magurran, 1988), was marginally nonsignificant (HB, treatment, P=0.07, Table 3).

Presence or absence of a fouling community

Treatment and sample date combined to have a significant effect on the number of *A. troschelii* (date by treatment interaction, $P<0.01$)—an effect contributing to the similar significant interaction effect for all species combined (Fig. 7, Table 4). Although the mean numbers of fish(es) were consistently higher at fouled FADs for *E. bipinnulata* alone and for all species minus *A. troschelii*, the only significant main effects were due to sample date (Table 4, Fig. 7). Species diversity (HB), though not richness (S), was significantly affected by sample date ($P=0.02$).

Artificial fish experiment

Experimental treatments (FADs with model fish, with lead weights or with nothing, Fig. 4) had no effect on any measured parameter—combined species, *A. troschelii* alone, *E. bipinnulata* alone, combined species less *A. troschelii*, species richness and diversity (Fig. 8, Table 5). All measures were significantly affected ($P<0.01$) by sample date except for *E. bipinnulata* alone (date, $P=0.48$). Although individual FADs varied in the number of associated *E. bipinnulata*,

Table 3

FAD-size effects and repeated measures ANOVA results (single FADs vs. triple-size FADs, n_1=n_2=12).

Dependent variable	Factor(s)	F	df	P	1–β
Number of of fishes	treatment	24.1	1, 6	<0.01	0.99
(all species combined)	date	10.7	2, 12	<0.01	0.97
	date × treatment	2.56	2, 12	0.12	0.41
Number of fish	treatment	10.9	1, 6	0.02	0.79
(*A. troschelii* only)	date	18.2	2, 12	<0.01	>0.99
	date × treatment	4.55	2, 12	0.03	0.66
Number of fishes	treatment	4.84	1, 6	0.07	0.45
(all spp–*A. troschelii*)	date	0.42	2, 12	0.67	0.10
	date × treatment	0.29	2, 12	0.75	0.09
Species richness (S)	treatment	11.3	1, 6	0.02	0.81
	date	0.38	2, 12	0.69	0.10
	date × treatment	0.38	2, 12	0.69	0.10
Species diversity (HB)	treatment	5.00	1, 6	0.07	0.46
	date	3.39	2, 12	0.07	0.52
	date × treatment	0.40	2, 12	0.68	0.10

these numbers were strikingly constant across sample date and, to a lesser extent, across treatments (Fig. 8).

Recruit-enriched vs. nonenriched FADs

Enriched FADs showed significantly higher rates of recruitment than nonenriched FADs: the regression line for the enriched FADs had a significant slope ($F_{(1,8)}$=20.76, P<0.01), but the regression line for the nonenriched FADs did not ($F_{(1,8)}$=2.29, P=0.17; Fig. 6). All additional fish were juvenile sergeant major damselfish, *Abudefduf troschelii*. These slopes are significantly different (t=3.05, 2 tailed test, v=6, P=0.02; Fig. 9); enriched FADs accumulated fish at a significantly higher rate (2.5 fish per hour) than did nonenriched FADs that accumulated fish at a rate of 0.1 fish per hour. Horizontal underwater visibility was 15 m at the beginning of the experiment.

Discussion

FAD size, the presence of a fouling community, and the presence of prior recruits all had positive effects on the size of FAD-associated assemblages, although the latter factor was assessed over a period of hours, whereas the former were assessed over days. The repeated removal of an existing assemblage also had significant effects due at least partially to treatment, but in all of these analyses sample date appeared to play the largest role in determining the numbers of fish(es) at these FADs. The presence of artificial fish or comparable-size weights did not significantly affect assemblage sizes. There was little support for the hypothesis that any of these factors might affect the species diversity of these assemblages; only species richness was significantly increased along with an increase in FAD size and this result may be an effect of assemblage size rather than object characteristics. Where treatment effects did significantly affect the numbers of fishes, their effects

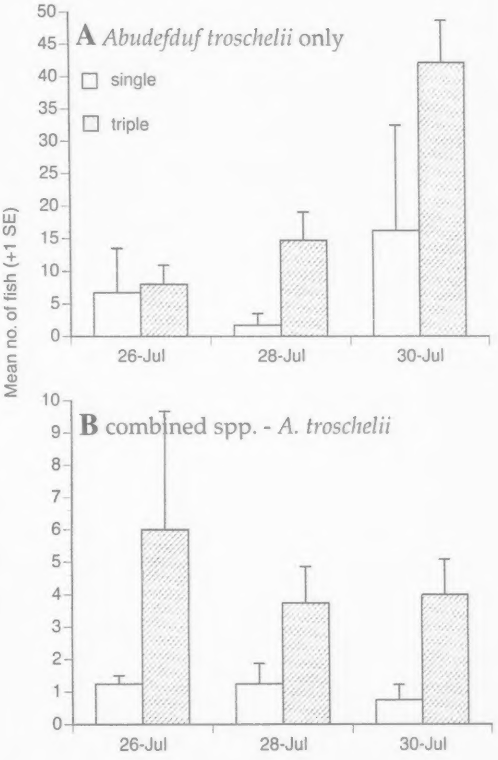


Figure 6

FAD-size effects (single FADs vs. triple-size FADs) on aggregation size (mean no. of fishes) for *Abudefduf troschelii* alone (**A**) and for all species combined less *A. troschelii* (**B**). See Tables 3–5 for sample sizes and ANOVA results for assemblage size and diversity measures.

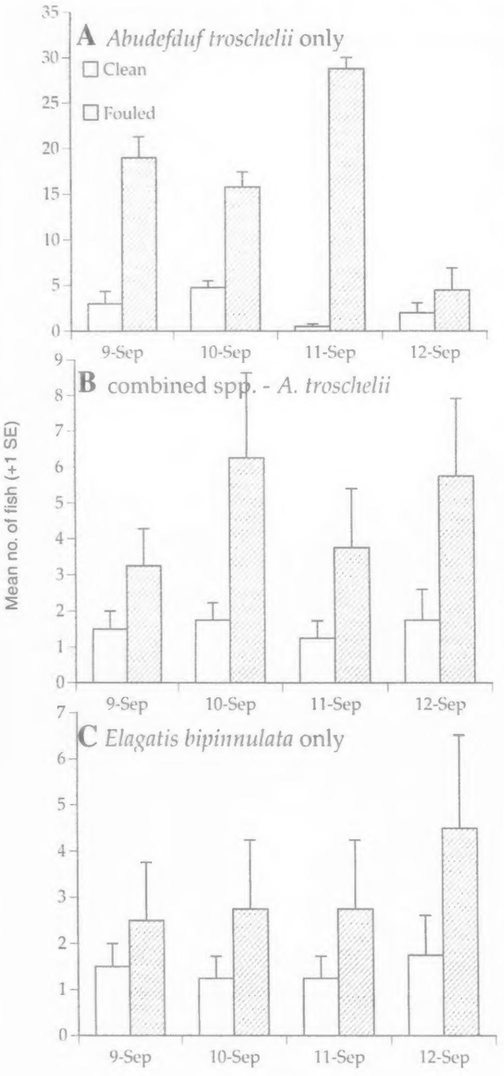


Figure 7

Effects of fouling community (fouled vs. unfouled [control]) on aggregation size (mean no. of fishes) for *Abudefduf troschelii* alone (**A**), for all species combined less *A. troschelii* (**B**), and for *Elagatis bipinnulata* alone (**C**). See Tables 3–5 for sample sizes and ANOVA results for assemblage size and diversity measures.

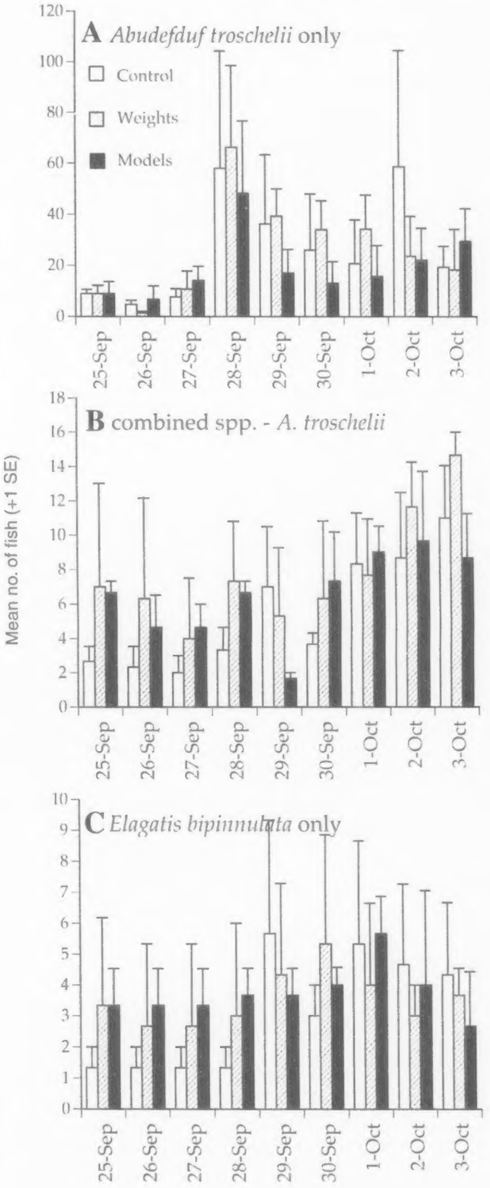


Figure 8

Artificial-fish effects (empty [control] FADs, FADs with weights, versus FADs with artificial fish) on aggregation size (mean no. of fishes) for *Abudefduf troschelii* alone (**A**), for all species combined less *A. troschelii* (**B**), and for *Elagatis bipinnulata* alone (**C**). See Tables 3–5 for sample sizes and ANOVA results for assemblage size and diversity measures.

on *Abudefduf troschelii* were generally the strongest. It is not clear whether this is a species-specific effect or if these results are due to the fact that *A. troschelii* was the most numerically important species.

The absence of a significant treatment main effect in the fish-removal experiments suggests that recruitment and loss from these anchored FADs is sufficiently rapid so that the complete removal of all fishes on a daily basis has no effect on the next day's assemblage size or diversity. These

Table 4

Repeated measures ANOVA results (absence of fouling community vs. presence of a fouling community, $n_1=n_2=16$) in fouling community experiment.

Dependent variable	Factor(s)	F	df	P	$1-\beta$
Number of fishes (all species combined)	treatment	39.8	1, 6	<0.01	0.99
	date	24.4	3, 18	<0.01	0.97
	date × treatment	33.5	3, 18	<0.01	0.41
Number of fish (*A. troschelii* only)	treatment	165	1, 6	<0.01	>0.99
	sate	19.7	3, 18	<0.01	>0.99
	date × treatment	25.2	3, 18	<0.01	>0.99
Number of fish (*E. bipinnulata* only)	treatment	1.06	1, 6	0.34	0.14
	date	4.89	3, 18	0.01	0.84
	date × treatment	2.15	3,18	0.13	0.45
Number of fishes (all spp.–minus *A. troschelii*)	treatment	3.01	1, 6	0.13	0.30
	date	3.88	3, 18	0.03	0.73
	date × treatment	2.27	3, 18	0.12	0.47
Species richness (S)	treatment	2.49	1, 6	0.17	0.26
	date	3.00	3, 18	0.06	0.60
	date × treatment	0.60	3, 18	0.62	0.15
Species diversity (HB)	treatment	1.74	1, 6	0.23	0.19
	date	4.35	3, 18	0.02	0.79
	date × treatment	1.91	3, 18	0.16	0.40

Table 5

Repeated measures ANOVA results (control [no weights and no artificial fish] vs. weights and vs. artificial fish, $n_1=n_2=n_3=27$) in the artificial fish experiment.

Dependent variable	Factor(s)	F	df	P	$1-\beta$
Number of fishes (all species combined)	treatment	0.13	2, 6	0.88	0.06
	date	3.52	8, 48	<0.01	0.97
	date × treatment	0.37	8, 48	0.98	0.20
Number of fish (*A. troschelii* only)	treatment	0.12	2, 6	0.89	0.06
	date	3.34	8, 48	<0.01	0.95
	date × treatment	0.41	8, 48	0.97	0.22
Number of fishes (all spp. minus *A. troschelii*)	treatment	0.29	2, 6	0.76	0.08
	date	4.82	8, 48	<0.01	>0.99
	date × treatment	0.73	8, 48	0.75	0.41
Number of fish (*E. bipinnulata* only)	treatment	0.05	2, 6	0.95	0.06
	date	0.95	8, 48	0.48	0.38
	date × treatment	0.36	8, 48	0.94	0.20
Species richness (S)	treatment	0.41	2, 6	0.68	0.10
	date	9.87	8, 48	<0.01	>0.99
	date × treatment	0.90	8, 48	0.58	0.50
Species diversity (HB)[1]	treatment	0.13	2, 5	0.88	0.06
	date	4.44	8, 47	<0.01	0.99
	date × treatment	1.00	8, 47	0.45	0.56

[1] Missing data were replaced according to the directions in Zar (1996), and the degrees of freedom were reduced accordingly.

results are consistent with those obtained by Wickham and Russell (1974). A similar result would occur if these FADs had a predictable carrying capacity and recruitment was sufficiently rapid that removal of the assemblage was followed by its replacement before the next observation. However, assemblage sizes within treatments varied widely from one day's observations to the next; therefore recruitment, rather than carrying capacity, seems to determine assemblage size.

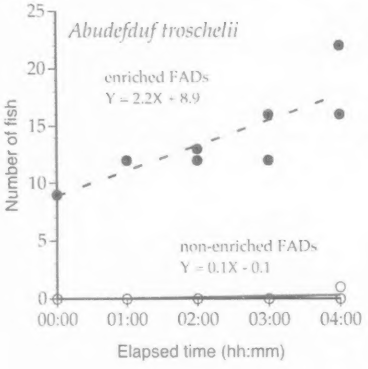


Figure 9

Changes in the number of fish associated with enriched (solid circles) and nonenriched (open circles) drifting FADs over time. Enriched FADs showed significantly higher rates of recruitment than did nonenriched FADs; the slopes of the regression lines are significantly different (t=3.05, 2-tailed test, v=6, P=0.02).

There appears to be insufficient time or stability for such factors as competition or predation to influence the size or diversity of these FAD-associated fish assemblages. Despite these results, individual fish do remain with a specific FAD for days: On at least five separate occasions associated with some of the other experiments described in this paper, individuals recognizable by scars and bite marks were sighted repeatedly as many as six days after the initial observation (Nelson, unpubl. data). Although the FADs and the associated fishes described in the present study are not directly comparable to FADs and fishes targeted in fisheries-scale operations, these experiments are among the first controlled efforts at understanding the effects of disturbance or fishing for FAD-associated assemblages.

The average assemblage size for all experiments and treatments varied considerably, often significantly, over time (Tables 2–5). Significant interaction effects between sample date and FAD treatments may be indicative of day-to-day recruitment fluctuations, dependent upon recruitment variability. A significant interaction may result when these effects are large and are in evidence regardless of the experimental treatment (i.e. occur in concert across treatments). Significant sample date effects (and series effects in the fish-removal experiment, Table 2) are likely a result of temporal fluctuations in the numbers of fishes available to recruit to the FADs.

Note that the two series in the fish-removal experiment differed not only in which FADs were given a particular treatment (positional effects), but also in time—the two series were necessarily run consecutively, not concurrently. I believe, however, it to be unlikely that positional effects influenced any of the results reported in the present study: treatments were assigned to FADs within the arrays in such a way as to ensure that inshore, offshore, or longshore positions were equally weighted among treatments. Significant series main effects, independent of additional factors, were found only for species richness and diversity (*HB*)—a result I attribute to changes in the availability of potential recruit species. Temporal patterns of juvenile reef fish recruitment are often variable and may be affected by such factors as spawning periodicity (Love et al., 1990), variable predation (Nelson, 2001), or changing physical oceanographic processes (Doherty, 1991; Levin, 1994; Kingsford and Finn, 1997). Rountree (1989), also, found that the mean numbers of the most abundant species observed around a FAD array off South Carolina varied widely during FAD deployment, albeit over a much longer time period (nearly 200 days). Thus, differences in assemblage size and diversity over time are not unexpected.

FAD size had significant, positive effects on assemblage size and species richness. Although tripling the FAD size resulted in a nearly threefold increase in the number of associated fishes (combined species), the response may not be linear. (Note, however, that Rountree (1989) demonstrated that the number of *Decapterus punctatus* associated with midwater FADs exhibited a significant, positive linear response to FAD size.) Further research will be necessary to resolve the effect of FAD size on numbers of aggregating fishes. Also of interest is the significant increase in species richness attributable to increased FAD size. Bortone et al. (1977) suggested that species diversity may be a function of "clump size" for *Sargassum*-associated fish assemblages, and Moser et al. (1998) found greater numbers of fish species under large (10–20 m diameter) mats of floating *Sargassum* than they did under smaller clumps (<1 m diameter) or in open water. However, the changes in species richness from this experiment could well be an effect of assemblage size; treatment effects on species diversity measured using the Brillouin index (*HB*) were marginally nonsignificant (Table 3, P=0.07). Significant sample date differences in treatment and evenness are due to large fluctuations in the abundance of the dominant species, *Abudefduf troschelii*, ranging at the triple-size FADs from 1 to 55 individuals over the course of 11 days.

Fishes were five times more numerous on average at fouled FADs than they were at comparable FADs lacking fouling organisms, but measures of diversity showed no significant treatment effect (Table 4). There was a significant interaction between treatment and sample date for the present experiment (Table 4) that may have been due to fluctuations in assemblage sizes among sample dates across both FAD treatments. The species composition of these assemblages was similar to that of other experiments, except that *Elagatis bipinnulata* were regularly observed: *Abudefduf troschelii* were the dominant species by abundance, followed by *E. bipinnulata*, and *Mugil* sp. All were small, young-of-the-year fishes (the largest *E. bipinnulata* individuals reached approximately 80 mm SL) and seemed not to be feeding on the larger invertebrates forming much of the colonizing community. During casual observations of FAD-associated fishes, I observed fish feeding on plankton carried past the FADs, but no physical contact with the FAD or fouling organisms. Ibrahim et al.

(1996) reported that none of the gut contents from FAD-associated fishes included sessile organisms found on their FADs (fish size ranges included specimens 8–14, 15–99, and ≥100 mm SL—the first two size categories are comparable to the fishes in the present study). Larger, piscivorous fishes do feed at least occassionally on smaller fishes associated with floating objects (Gooding and Magnuson, 1967), but published gut content studies are conflicting. Some suggest that piscivorous species that associate with flotsam rely on other sources of food (e.g. Gooding and Magnuson, 1967; Hunter and Mitchell, 1967; Brock, 1985), while others suggest that flotsam- (or algae-) associated fishes form an important food resource for these larger piscivorous fishes (Dooley, 1972; Manooch et al., 1984; Coston-Clements et al., 1991). Morgan et al. (1985) noted the occurrence of at least two members of the *Sargassum*-associated invertebrate fauna among the stomach contents of several species of pelagic fishes. From the perspective of flotsam- or FAD-associated fishes, opportunistic predation by piscivores that do not associate with FADs may be more important than predation by other members of the assemblage. Additional gut content data from juvenile and nonpiscivorous fishes are sorely lacking. I address possible explanations for the results of the present study below.

I recorded no significant treatment effect attributable to differences between FADs with artificial fish, FADs deployed with artificial-fish–sized weights, or control FADs. I attribute significant sample date effects to day-to-day changes in constituent individuals and the fluctuating availability of potential recruits. Numbers of *E. bipinnulata* were strikingly constant across treatments and sample dates in this experiment (Table 5, Fig. 8) and in the fouling experiment (Fig. 7) and seemed to indicate an apparently unusual characteristic of this species—individuals remaining associated with a given FAD for multiple days. Although the experiment was intended to distinguish between FADs with prior recruits versus FADs without prior recruits, the lack of a significant treatment effect does not negate the possibility that potential recruits would distinguish between occupied and unoccupied FADs. The painted artificial fish and lead weights clearly lacked many attributes of living fish. However, comparable numbers of recruits found at all treatments suggest that a change in the structural complexity of the FADs did not affect assemblage size or diversity. Although the addition of four small lead weights (artificial fish were painted and oriented differently but were still lead weights) did not appear to increase appreciably the visible surface area of those FADs, the subsequent experiment with live fishes instead of artificial fish had a dramatic effect on recruitment; therefore sizeable changes in the physical size of a FAD may be necessary to yield a response in fish recruitment. The potential roles of structural complexity and orientation of FADs will be informative areas for future research. Past investigations in these areas (e.g. Hunter and Mitchell, 1968; Klima and Wickham, 1971; Wickham et al., 1973) have provided a useful beginning, but more work is needed.

Although sample sizes were small, the presence of prior *Abudefduf troschelii* "recruits" (enriched FADs) had a significant effect on patterns of subsequent recruitment; this effect contrasted sharply with FADs lacking fish at the start of this experiment (nonenriched FADs). For this species, these results point to a social aspect to these aggregations, and sociality may also be involved in the recruitment of other species, particularly the schooling fishes *Caranx* spp., *Polydactylus approximans,* and *Mugil* spp., as suggested for some scombrids (e.g. Dagorn and Fréon, 1999). The addition of fishes below a FAD may increase recruitment rates by rendering the object more visible, although the artificial fish experiment indicated that simply adding fish-size objects beneath a FAD does not affect recruitment. Comparisons between these two experiments are tenuous, however, because the artificial fish experiment employed anchored FADs observed over a period of days, whereas the enriched FAD experiment used drifting FADs observed over a course of hours.

Why do FAD size, the presence of a fouling community, and the actual presence of prior recruits at a FAD each have the effect of increasing the size and, possibly, the diversity of FAD-associated assemblages of juvenile fishes? The simplest explanation is that these factors contribute to the target strength of the object, increasing the visual, olfactory, or auditory stimulus (or some combination) of the floating object. Larger objects should be easier to find, especially if potential recruits rely on vision to explore their environment. Kellison and Sedberry (1998) found that the fishes associated with mid-water floating structures that were tethered to an artificial reef decreased in abundance over time (193 days), and suggested that the loss in buoyancy associated with the development of a fouling community may have reduced the effective size of these floating objects, accounting for fewer associated fishes (see also Hunter and Mitchell, 1968; Rountree, 1989). To account for the positive effects of a fouling community observed in the present study, it seems reasonable to suppose that fouling organisms may be detected by olfactory means; Sweatman (1988) has shown that some larval fishes use olfactory cues for settlement on reefs. Further experiments, for example experiments controlling for FAD size, odor cues, and visibility of the FADs, are needed to determine why some of these factors exhibit these effects.

Future research on the role of flotsam as shelter from predators and as a conveyance to suitable habitat could yield evolutionary explanations for the attraction to floating objects. For these small fishes, such objects likely represent a shelter from predators (Mitchell and Hunter, 1970). Some species do respond to the approach of an observer by positioning themselves so that the FAD is between them and the observer. Particularly during daylight and crepuscular hours when visually-oriented predators are most active, flotsam may offer refuge in a habitat where there is little alternative refuge. During the day, when onshore winds drive drifting objects towards shallow water, flotsam and drift algae, unlike anchored FADs, may also offer a comparatively safe conveyance to more suitable habitat. Thus, there may be adaptive advantages for juvenile reef fishes in associating with floating objects.

Although the juvenile fishes associated with the FADs used in the present study are not of interest to any fishery, the patterns observed from them may be relevant to FADs deployed commercially to aggregate fish species at various life history stages. FAD size is clearly relevant to those interested in studying potential improvements to FAD design. Carefully controlled studies on the importance of surface area versus volume and the orientation of FAD structures are needed. The role of a fouling community, too, deserves further investigations. Although a fouling community may weigh down streamers (trailing pieces of buoyant material intended to increase the subsurface of area of a FAD), such a community may also improve recruitment and possibly retention of recruits around a FAD. Finally, the importance of the initial recruits to a floating object should be studied further. Enriching a FAD may increase the speed at which additional fishes are recruited. Improved artificial fish may prove more effective than the items used in the present research. FADs are an important tool in a number of artisanal (small-scale fishery based on traditional methods), sport, and commercial fisheries, especially in tropical waters where FAD fisheries particularly target tunas (Scombridae), jacks (Carangidae), and *Coryphaena* spp. (Galea, 1961; Klima and Wickham, 1971; Beets, 1989; Hilborn and Medley, 1989; Friedlander et al., 1994; Higashi, 1994; Hall et al., 1999b). Due largely to the potential for fisheries enhancement, considerable research has been focused on the importance of floating-object characteristics and the numbers of fishes attracted to such objects; however, the results have been difficult to interpret and are often conflicting (Rountree, 1989; Kingsford, 1993; Druce and Kingsford, 1995). Because log sets in tuna purse-seine fisheries (where fishermen target fish associated with drifting logs or FADs) are associated with high levels of bycatch (Hall, 1998; Lennert-Cody and Hall, 2000), the behavior and ecology of flotsam-associated species is in urgent need of study so that a means of reducing bycatch may be devised.

This study made use of FADs floating at the surface; studies by other researchers have employed similar tools or they have used FADs tethered in mid-water. No one has examined the effects of FAD position in relation to the surface, and the implicit assumption appears to be that there is no biologically significant difference. This assumption has not been tested, although comparisons between data from floating structures, whether at the surface, mid-water, or tethered close to the bottom, are common in the literature. I have made comparisons between my data from surface FADs and results from mid-water FADs (e.g. Wickham and Russell, 1974; Rountree, 1990); such comparisons may be misleading and should be interpreted with caution.

The results from the present study indicate that turnover rates at nearshore anchored FADs are high and that undisturbed FAD assemblages may show little difference in these rates from disturbed FADs. Fishes recruiting to these FADs discriminate among potential floating objects, forming larger, more species-rich assemblages around triple-size FADs than around single FADs. FADs possessing a fouling biota also attract larger (though no more diverse) assemblages than do clean FADs. The latter effect was complicated by temporal fluctuations that overlay these treatment effects, resulting in day-to-day changes in the total numbers of fishes in both treatments (Table 4, Fig. 7). Further, the presence of prior recruits in the enrichment experiment had a strong effect on subsequent recruitment. Thus, the association of juvenile fishes with floating objects is not a haphazard process, and floating-object characteristics play potentially important roles in fish recruitment to these objects. These results suggest that associating with flotsam may be adaptive, rather than an accidental behavior and support Kingsford's hypothesis (Kingsford, 1993) that floating material is an important environmental component in the relationship between environment and some juvenile fishes.

Acknowledgments

For help in the field, I am grateful to I. Nelson and D. Mansue. W. L. Montgomery, S. Shuster, and to two anonymous reviewers who provided helpful criticism. Funding was provided by the American Museum of Natural History (Lerner-Gray Fund), American Society of Ichthyologists and Herpetologists (Raney Award), Animal Behavior Society, International Women's Fishing Association (Max Coan Memorial Scholarship), Seaspace/Houston Underwater Club, Smithsonian Tropical Research Institute (STRI), and Sigma Xi. D. R. Robertson of STRI and D. Margulise, R. Olson, and V. Scholey of the Inter-American Tropical Tuna Commission provided invaluable advice and logistical support.

Literature cited

Beets, J.
1989. Experimental evaluation of fish recruitment to combinations of fish aggregating devices and benthic artificial reefs. Bull. Mar. Sci. 44:973–983.

Bortone, S., P. A. Hastings, and S. B. Collard.
1977. The pelagic-*Sargassum* ichthyofauna of the eastern Gulf of Mexico. Northeast Gulf Sci. 1:60–67.

Brock, R. E.
1985. Preliminary study of the feeding habits of pelagic fish around Hawaiian fish aggregation devices or can fish aggregation devices enhance local fisheries productivity? Bull. Mar. Sci. 37:40–49.

Coston-Clements, L., L. R. Settle, D. E. Hoss, and F. A. Cross.
1991. Utilization of the *Sargassum* habitat by marine invertebrates and vertebrates—a review. NOAA Tech. Memo. NMFS-SEFSC-296:32.

Crawford, R. E., and J. K. Jorgenson.
1993. Schooling behaviour of arctic cod, *Boreogadus saida*, in relation to drifting pack ice. Environ. Biol. Fish. 36: 345–357.

Dagorn, L., and P. Fréon.
1999. Tropical tuna associated with floating objects: a simulation study of the meeting point hypothesis. Can. J. Fish. Aquat. Sci. 56:984–993.

Doherty, P. J.
1991. Spatial and temporal patterns in recruitment. *In* The ecology of fishes on coral reefs (P. F. Sale, ed.), p. 261–294. Academic Press, San Diego, CA.

Dooley, J.
1972. Fishes associated with the pelagic *Sargassum* complex, with a discussion of the *Sargassum* community. Contrib. Mar. Sci. 16:1–32.

Druce, B. E., and M. J. Kingsford.

1995. An experimental investigation on the fishes associated with drifting objects in coastal waters of temperate Australia. Bull. Mar. Sci. 57:378–392.

Fedoryako, B. I.

1989. A comparative characteristic of oceanic fish assemblages associated with floating debris. J. Ichthyol. 29: 128–137.

Friedlander, A., J. Beets, and W. Tobias.

1994. Effects of fish aggregating device design and location on the fishing success in the U.S. Virgin Islands. Bull. Mar. Sci. 55:592–601.

Gaertmer, D., and M. Medina-Gaertner.

1999. An overview of the relationship between tunas and floating objects in the south of Caribbean Sea. IATTC (Inter-American Tropical Tuna Commission), Spec. Rep. 11:66–86.

Galea, J. A.

1961. The "Kannizzati" fishery. Gen. Fish. Counc. Mediterr. Sess. Rep. 6:85–91.

Gooding, R. M., and J. J. Magnuson.

1967. Ecological significance of a drifting object to pelagic fishes. Pac. Sci. 21:486–497.

Hall, M. A.

1998. An ecological view of the tuna-dolphin problem: impacts and trade-offs. Rev. Fish Biol. Fish. 8:1–34.

Hall, M. A., D. L. Alverson, and K. I. Metuzals.

2000. By-catch: problems and solutions. Mar. Pollut. Bull. 41:204–219.

Hall, M. A., M. Garcia, C. Lennert-Cody, P. Arenas, and F. Miller.

1999a. The association of tunas with floating objects and dolphins in the eastern Pacific Ocean: a review of the current purse-seine fishery. IATTC, Spec. Rep. 11:87–194.

Hall, M. A., C. Lennert-Cody, M. Garcia, and P. Arenas.

1999b. Characteristics of floating objects and their attractiveness for tunas. IATTC, Spec. Rep. 11:396–446.

Hallier, J. P., and J. I. Parajua.

1999. Review of tuna fisheries on floating objects in the Indian Ocean. IATTC, Spec. Rep. 11:195–221.

Hampton, J., and K. Bailey.

1999. Fishing for tunas associated with floating objects: review of the western Pacific fishery. IATTC, Spec. Rep. 11:222–284.

Higashi, G. R.

1994. Ten years of fish aggregating device (FAD) design development in Hawaii. Bull. Mar. Sci. 55:651–666.

Hilborn, R., and P. Medley.

1989. Tuna purse-seine fishing with fish-aggregating devices (FAD): models of tuna FAD interactions. Can. J. Fish. Aquat. Sci. 46:28–32.

Hunter, J. R., and C. T. Mitchell.

1967. Association of fishes with flotsam in the offshore waters of Central America. Fish. Bull. 66:13–29.

1968. Field experiments on the attraction of pelagic fish to floating objects. J. Cons. Int. Explor. Mer 31:427–434.

Ibrahim, S., M. A. Ambak, L. Shamsudin, and M. Z. Samsudin.

1996. Importance of fish aggregating devices (FADs) as substrates for food organisms of fish. Fish. Res. 27:265–273.

Kellison, G. T., and G. R. Sedberry.

1998. The effects of artificial reef vertical profile and hole diameter on fishes off South Carolina. Bull. Mar. Sci. 62: 763–780.

Kingsford, M. J.

1992. Drift algae and small fish in coastal waters of northeastern New Zealand. Mar. Ecol. Progr. Ser. 80:41–55.

1993. Biotic and abiotic structure in the pelagic environment: importance to small fishes. Bull. Mar. Sci. 53:393–415.

1995. Drift algae: a contribution to near-shore habitat complexity in the pelagic environment and an attractant for fish. Mar. Ecol. Progr. Ser. 116:297–301.

Kingsford, M. J., and M. Finn.

1997. The influence of phase of the moon and physical processes on the input of presettlement fishes to coral reefs. J. Fish Biol. 51:176–205.

Klima, E. F., and D. A. Wickham.

1971. Attraction of coastal pelagic fishes with artificial structures. Trans. Am. Fish. Soc. 1:86–99.

Kojima, S.

1960. Fishing for dolphins in the western part of the Japan Sea. V. Species of fishes attracted to bamboo rafts. Bull. Jpn. Soc. Sci. Fish. 26:379–382.

Lennert-Cody, C. E., and M. A. Hall.

2000. The development of the purse seine fishery on drifting fish aggregating devices in the eastern Pacific Ocean: 1992–1998. *In* Pêche thonière et dispositifs de concentration de poissons: colloque DCP, Martinique, Octobre 1999, p. 78–107. Institut Français de Recherche pour l'Exploitation de la Mer, Issy-les-Moulineaux, France.

Levin, P. S.

1994. Fine-scale temporal variation in recruitment of a temperate demersal fish: the importance of settlement versus post-settlement loss. Oecologia 97:124–133.

Love, M. S., M. H. Carr, and L. J. Haldorson.

1990. The ecology of substrate-associated juveniles of the genus *Sebastes*. Environ. Biol. Fish. 30:225–243.

Magurran, A. E.

1988. Ecological diversity and its measurement, 179 p. Princeton Univ. Press, Princeton, NJ.

Manooch, C. S., III, D. L. Mason, and R. S. Nelson.

1984. Food and gastrointestinal parasites of dolphin *Coryphaena hippurus* collected along the southeastern and Gulf coasts of the United States. Bull. Jpn. Soc. Sci. Fish. 50: 1511–1525.

Massuti, E., S. Deudero, P. Sanchez, and B. Morales-Nin.

1998. Diet and feeding of dolphin (*Coryphaena hippurus*) in western Mediterranean waters. Bull. Mar. Sci. 63: 329–341.

McCleneghan, K., and J. L. Houk.

1978. A diver-operated net for catching large numbers of juvenile marine fishes. Calif. Fish Game 64:305–307.

Mitchell, C. T., and J. R. Hunter.

1970. Fishes associated with drifting kelp, Macrocystis pyrifera, off the coast of southern California and northern Baja California. Calif. Fish Game 56:288–297.

Morgan, S. G., C. S. Manooch III, D. L. Mason, and J. W. Goy.

1985. Pelagic fish predation on *Cerataspis*, a rare larval genus of oceanic penaeoids. Bull. Mar. Sci. 36:249–259.

Moser, M. L., P. J. Auster, and J. B. Bichy.

1998. Effects of mat morphology on large *Sargassum*-associated fishes: observations from a remotely operated vehicle (ROV) and free-floating video camcorders. Environ. Biol. Fish. 51:391–398.

Nelson, P. A.

1999. The ecology and behavior of flotsam-associated marine fish aggregations. Ph.D. diss., 137 p. Northern Arizona Univ., Flagstaff, AZ.

2001. Behavioral ecology of young-of-the-year kelp rockfish, *Sebastes atrovirens* Jordan and Gilbert (Pisces: Scorpaenidae). J. Exp. Mar. Biol. Ecol. 256:33–50.

Parin, N. V., and B. I. Fedoryako.
1999. Pelagic fish communities around floating objects in the open ocean. IATTC, Spec. Rep. 11:447–458.

Rountree, R. A.
1989. Association of fishes with fish aggregation devices: effects of structure size on fish abundance. Bull. Mar. Sci. 44:960–972.
1990. Community structure of fishes attracted to shallow water fish aggregation devices off South Carolina, U.S.A. Environ. Biol. Fish. 29:241–262.

Safran, P.
1990. Drifting seaweed and associated ichthyofauna: floating nursery in Tohoku waters. Mer 28:225–239.

Safran, P., and M. Omori.
1990. Some ecological observations on fishes associated with drifting seaweed off Tohoku coast, Japan. Mar. Biol. 105:395–402.

Suzuki, Z.
1999. Distribution of floating logs in the Pacific and purse seine sets on tunas associated with logs by Japanese boats in the tropical western and central Pacific. IATTC, Spec. Rep. 11:459–479.

Sweatman, H. P. A.
1988. Field evidence that settling coral reef fish larvae detect resident fishes using disolved chemical cues. J. Exp. Mar. Biol. Ecol. 124:163–174.

Wickham, D. A., and G. M. Russell.
1974. An evaluation of mid-water artificial structures for attracting coastal pelagic fishes. Fish. Bull. 72:181–191.

Wickham, D. A., J. W. Watson Jr., and L. H. Ogren.
1973. The efficacy of midwater artificial structures for attracting pelagic sport fish. Trans. Am. Fish. Soc. 3:563–572.

Zar, J. H.
1996. Biostatistical analysis, 3rd ed., 662 p. Prentice Hall, Upper Saddle River, NJ.

Age and growth of the bastard grunt (*Pomadasys incisus*: Haemulidae) inhabiting the Canarian archipelago, Northwest Africa

José G. Pajuelo

José M. Lorenzo

Departament of Biology
University of Las Palmas de Gran Canaria
Campus Universitario de Tafira
35017 Las Palmas de Gran Canaria, Spain
E-mail address (for J. G. Pajuelo): jpaguelo@dbio.ulpgc.es

Muriel Gregoire

Faculty of Biology
University of Liége
Liége, Belgium
Present address: Departament of Biology
University of Las Palmas de Gran Canaria
Campus Universitario de Tafira
35017 Las Palmas de Gran Canaria, Spain

Abstract—The bastard grunt (*Pomadasys incisus*) is one of the most abundant coastal demersal fishes inhabiting the Canary Islands. Age and growth were studied from samples collected between October 2000 and September 2001. Growth analysis revealed that this species is a fast growing and moderately short-lived species (ages up to seven years recorded). Length-at-age was described by the von Bertalanffy growth model (L_∞=309.58 mm; k=0.220/year; t_0=−1.865 year), the Schnute growth model (y_1=126.66 mm; y_2=293.50 mm; a=−0.426; b= 5.963), and the seasonalized von Bertalanffy growth model (L_∞=309.93 mm; k=0.218/year; t_0= −1.896 year; C=0.555; t_s=0.652). Individuals grow quickly in their first year, attaining approximately 60% of their maximum length; after the first year, their growth rate drops rapidly as energy is probably diverted to reproduction. The parameters of the von Bertalanffy weight growth curve were W_∞=788.22 mm; k=0.1567/year; t_0= −1.984 year. Fish total length and otolith radius were closely correlated, r^2=0.912. A power relationship was estimated between the total length and the otolith radius (a=49.93; v=0.851). A year's growth was represented by an opaque and hyaline (translucent) zone—an annulus. Backcalculated lengths were similar to those predicted by the growth models. Growth parameters estimated from the backcalculated sizes at age were L_∞=315.23 mm; k=0.217/year; and t_0= −1.73 year.

The family Haemulidae consists of 16 genera (126 species), including the genus *Pomadasys.* This genus is represented by 37 species distributed around the world. Members of this family are commonly referred to as grunt (Bauchot and Huraeu, 1990). Only the bastard grunt (*Pomadasys incisus* (Bowdich, 1825)) is found off the Canary Islands. The bastard grunt is a coastal demersal fish species inhabiting marine and brackish waters along the eastern central Atlantic coasts from the Strait of Gibraltar to Angola, and also in the Canaries, Azores, and Cape Verde Islands (Bauchot and Hureau, 1990). In the Canary Islands, where the bastard grunt is one of the most abundant species, it has been observed in high densities in schools along coastal waters.

Information on the biology of the bastard grunt is not available anywhere in the world. Despite its widespread occurrence, the bastard grunt has no commercial value for its low quality meat and it is discarded in the Canarian archipelago. The need for a biologically based discard management strategy and the paucity of data available on the biology of this species have prompted an investigation into aspects of its life history. We report aspects of age and growth, which are important parameters in models for managing the population of the bastard grunt off the Canary Islands.

Materials and methods

A total of 878 individuals of *P. incisus* were collected at weekly intervals from discarded commercial catches taken between October 2000 and September 2001 off Gran Canaria (Canary Islands, central-east Atlantic, 27°57′24″N–15°35′23″W).

Each fish was measured to the nearest mm for total length (L_t) and weighed to the near 0.1 g for total body weight (W_t). Sex was assessed visually and sagittal otoliths were removed, cleaned, and stored dry for later age determination.

Age estimation was made by identifying and counting annuli following Williams and Bedford (1974). An annulus was defined as a hyaline zone formed annually in the winter season when there is low growth and an opaque zone formed annually in the summer season when there is increased growth. The whole otoliths were placed in a blackened bottom watch glass containing water and examined under a compound microscope (10×) with reflected light. Counts of the growth bands were made

Manuscript approved for publication 19 June 2003 by Scientific Editor.

Manuscript received 26 June 2003 at NMFS Scientific Publications Office.

Fish. Bull. 101:851–859 (2003).

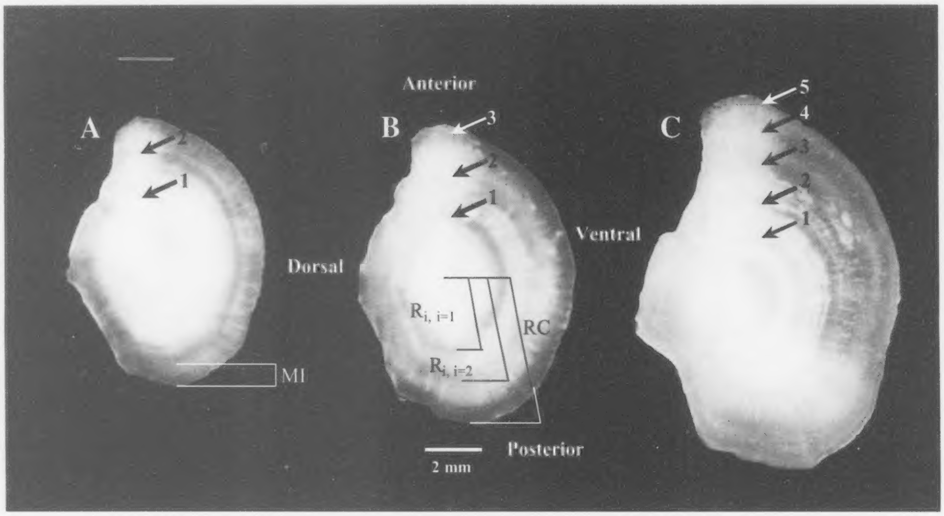


Figure 1

Saggital otoliths of *P. incisus* collected off the Canary Islands: (**A**) from a two-year-old (182 mm); (**B**) from a three-year-old (202 mm); and (**C**) from a five-year-old (265 mm) fish. *Ri* is the radius of the i^{th} band (distance from the centre of the otolith to the outer margin of the annulus), *Rc* is the radius of the otolith at capture, and MI is the marginal increment measured.

by two readers without knowledge of the size and sex of the specimens, or previous counts of the other reader. Counts were made for otoliths of each individual on two separate occasions, and only coincident readings were accepted. The same approach was used to determine the final number of bands in each specimen, and a consensus was reached between readers on the final counts. Reproducibility of the resultant age estimates was evaluated with the coefficient of variation (CV) (Chang, 1982) and the index of average percent error (IAPE) (Beamish and Fournier, 1981).

To validate that rings were formed annually in the bastard grunt, we analyzed the monthly mean marginal increment (Hyndes and Potter, 1997). Marginal increment, estimated as the distance between the outer edge of the outermost annual ring and the periphery of each otolith, was measured (to the nearest 0.01 mm) with an ocular micrometer (Fig. 1). Measurements were always made along the longest axis of the otolith. The pattern expected in the marginal increment would be a minimal value at the start of the growth period, increasing with time until the measurement fell to a minimum again at the formation of the next period of growth (Pilling et al., 2000). The size of the marginal increment varies both with the age of the fish and the time of sampling during the year. Because older fish grow slower than younger fish, a smaller marginal increment is expected. For this reason, to assess the possibility of false annulus formation among either younger or older bastard grunt, quantitative marginal increment analyses should be standardized for age. Therefore, we used age class to standardize our analyses. Owing to the wide range of ages encountered, however, there were insufficient samples to fully accomplish this standardization. It was necessary to combine the ages in two or more age groups representing fast, moderate, and slow-growing individuals (Pilling et al., 2000). Mean marginal increments were plotted against month of capture, and the minimum was used to indicate the month of annulus formation.

Once the periodicity and timing of ring formation were verified, the age of each fish was determined from the number of annuli, the assumed birthdate, and the sampling date. It was assumed that annulus formation began 1 January, corresponding to the peak of spawning in the species (Gregoire[1]). The difference between the date of capture and the birthdate was used to estimate a fractional age (Gordoa and Molí, 1997). This fraction was added to the number of annuli read in the otoliths to avoid any potential bias in growth estimates due to differences in sampling date (Gordoa and Molí, 1997).

Length-at-age was described by the three-parameter specialized von Bertalanffy growth model (Ricker, 1973):

$$L_t = L_\infty(1 - e^{-k(t-t_0)}),$$

the four-parameter Schnute growth model (Schnute, 1981):

---

[1] Gregoire, M. 2001. Edad y crecimiento del roncador *Pomadasys incisus* (Bowdich, 1825) de Gran Canaria (Islas Canarias). Unpubl. data. ULPGC Socrates/Erasmus Research Report, 34 p. Departament of Biology, University of Las Palmas de Gran Canaria. 35017 Las Palmas de Gran Canaria. Spain.

$$L_t = \left[y_1^b + (y_2^b - y_1^b)\left(\frac{1-e^{a(t-A_1)}}{1-e^{-a(A_2-A_1)}} \right) \right]^{\frac{1}{b}},$$

and the seasonalized von Bertalanffy growth model (Pitcher and Macdonald, 1973)

$$L_t = L_\infty \left(1 - e^{-k(t-t_0)-\left(\frac{Ck}{2\pi}\sin(2\pi(t-t_s))\right)} \right),$$

where A_1 = the smallest age in the sample;
A_2 = the largest age in the sample;
T_0 = the age at zero length;
L_t = the length-at-age;
L_∞ = the predicted asymptotic length;
y_1 = the estimated mean length of A_1-year-old fish;
y_2 = the estimated mean length of A_2-year-old fish;
C = the amplitude of the fluctuation in seasonal growth;
t_s = the point of the minumum growth (t_s=WP+0.5); and
k = the Brody growth constant (Schnute, 1981; Sparre and Venema, 1995).

The models were fitted to data with the Marquardt's algorithm for nonlinear least squares parameter estimation (Gayanilo et al., 1996). A nonparametric, one-sample test was applied to test for residual randomness and a Bartlett's test was used to test for their homoscedasticity. Von Bertalanffy growth model parameters were also estimated for observed W_t as a function of age by substituting weights in place of lengths in the growth equation and incorporating b derived by the weight-length regression (Sparre and Venema, 1995):

$$W_t = W_\infty(1-e^{-k(t-t_0)})^b,$$

where W_t = the weight at age; and
W_∞ = the predicted asymptotic weight.

Backcalculated size of each fish at the time of formation of each annulus was determined by a backcalculation formula consistent with the body proportional hypothesis (Campana, 1990; Francis, 1990). The measurements were the following: the radius of the i^{th} band (Ri, 0.01 mm, distance from the center of the otolith to the outer margin of the annulus) and the radius of the otolith at capture (Rc, 0.01 mm, distance from the center of the otolith to the periphery). These measurements were always made along the longest axis of the otolith (Fig. 1). The relationship between the radius of the otolith at capture (Rc) and the total length was estimated as a power function (nonlinear relationship). It is estimated by fitting the data by a regression of log(L_t) on log(Rc) consistent with the body proportional hypothesis (BPH). The length of an individual when the i^{th} band was laid down (Li, mm) was calculated as

$$Li = (Ri/Rc)^v Lc,$$

where Lc = the length-at-capture; and
v = a constant derived from the relationship of total length to otolith radius (Francis, 1990).

The von Bertalanffy growth curve was fitted to the backcalculated length-at-age by means of Marquardt's algorithm for nonlinear least squares parameter estimation (Gayanilo et al., 1996).

Results

Of the 878 fish examined, 377 (42.9%) were males and 412 (46.9%) females. The remaining 89 (10.1%) individuals were immature and could not be identified macroscopically. Fish varied in size from 103 to 304 mm L_t, and weighed between 8.7 and 137.1 g W_t. Males varied from 143 to 298 mm L_t, and total mass was from 9.3 to 114.7 g W_t. Female total length varied between 134 and 304 mm and total mass was from 13.2 to 137.1 g. Immature fish varied from 103 to 186 mm L_t and from 8.7 to 29.6 g W_t. No significant differences were found between males and females in mean size (Student's t-test, $t=1.03<t_{0.05,787}=1.65$) or weight (Student's t-test, $t=1.57<t_{0.05,787}=1.65$).

Sagittae of bastard grunt are ovate and laterally compressed. Annuli were clearly differentiated under reflected light on a black background: the opaque zones are milky in appearance and the hyaline zones relatively transparent (Fig. 1). The number of annuli counted for each individual were similar for the two readers. Of the 878 otoliths examined, 22 (2.5%) were rejected as unreadable, three were completely translucent, eight were broken, and 11 had poorly defined growth zones. Of the remaining 856 otoliths, consensus was reached for 812 (94.8%). The index of average percent error (IAPE) of band counts for each reader did not differ greatly, and was slighly lower for the first author (2.47) than for the second (2.69). The precision of the age estimates was also expressed as the CV as recommended by Campana (2001). The level of precision was approximately 5% for both readers.

Marginal increments were measured for 812 individuals. In the otoliths with one and two annuli (fast-growing young individuals), the lowest monthly mean marginal increments were recorded in January and increased throughout the year (Fig. 2). The marginal increments in the otoliths with three and more than three annuli (moderate and slow-growing individuals) also followed a similar trend. Thus, irrespective of the age of the fish, the marginal increments declined markedly and then rose progressively only once during a 12-month period. Therefore, only one annulus is formed in December–February.

Alternating opaque and hyaline zones were clearly visible on the otoliths. Up to seven marks, assumed to be annuli, were visible in the otoliths sampled. Two- and three-year-old fish were the dominant age classes and over 80% of fish were three years old or less (Table 1). Over 45% of the growth was achieved by the end of the first year. By the end of the second year, fish had attained 60% of the mean observed length.

The three growth models provided a good fit to the data (Table 2, Fig.3). Length-at-age was adequately described by the three models with the statistically suitable absolute error structure because it provided both residual randomness and homoscedasticity. The computed value of 0.15 for the winter point of the seasonalized von Bertalanffy growth model indicated that the lowest growth rate occurred at about two months after the birth date, meaning that the growth rate was reduced in winter.

No significant difference was found between the von Bertalanffy growth models with a likelihood ratio test ($F=0.15<F_{0.05;2;1560}$), and a significant difference was found between the von Bertalanffy growth models and the Schnute growth model with the same test ($F>F_{0.05;2;1560}$); therefore, the specialized von Bertalanffy growth model was chosen because it has fewer parameters making it statistically more robust, its parameters are commonly used in mortality estimates and per recruit modelling, and because it allows for comparison between growth studies conducted on other species (Booth, 1997). No significant differences were found between mean lengths-at-age of males and females with a Student's t-test ($t=0.52<t_{0.05,787}=1.65$) or between the von Bertalanffy growth curves for separate sexes with a Hotelling's T^2 test ($T^2=4.73<T_0{}^2{}_{0.05,3,784}=7.89$). The data were pooled as a single growth model. In fitting the growth curve to the age-length data, age 0 was disregarded because this age group was not well represented. The von Bertalanffy growth curve derived from the observed weight at age for males and females was not significantly different (Hotelling's T^2-test, $T^2=6.91<T_0{}^2{}_{0.05,3,784}=7.89$; Fig. 4).

Fish total length and otolith radius were closely correlated (Fig. 5). The value of the derived constant ($v=0.851$) was different from one (Student t-test; $t=14.61>t_{0.05,810}=1.65$). Backcalculated size at time of annulus formation was used to provide length-at-age data unbiased by differences in sampling date and to estimate the von Bertalanffy equation (Table 3). Backcalculated lengths were similar to those predicted by the growth models. Growth parameters estimated from the backcalculated sizes at age were $L_\infty=315.23$ mm; $k=0.217$/year; and $t_0=-1.733$ year. The data was pooled as a single growth model because no significant differences in the growth parameters were found between males and females (Hotelling's T^2-test, $T^2=48.3>T_0{}^2{}_{0.05,3,784}=7.89$).

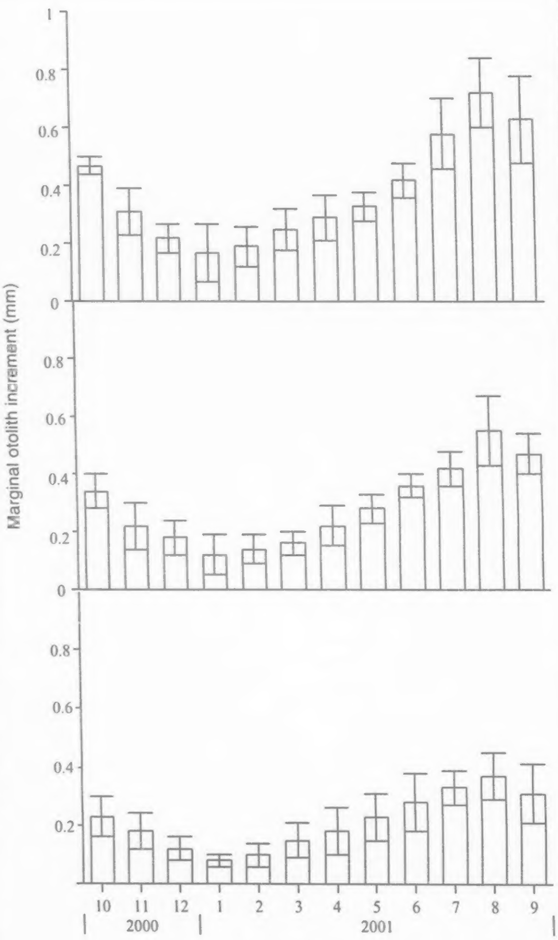


Figure 2

Mean monthly marginal increment from otoliths with one and two, three, and more than three annuli, representing fast, moderate, and slow-growing individuals of *P. incisus* off the Canary Islands. Standard errors are identified by the bars.

Discussion

Age estimation in fishes is complicated by the phenomenon of "stacking" of growth zones towards the otolith margin, particularly in older fish (Buxton and Clarke, 1991). In many cases age determination is difficult because whole otoliths are so thick that light does not pass through (Buxton and Clarke 1991); however, in the bastard grunt off the Canary Islands the translucency of the otoliths allows aging with relative ease. The values of the IAPE and the CV suggested that the precision levels obtained are according to the reference point values indicated by Campana (2001). The oldest age estimate obtained in the present study was seven years and the phenomenon of stacking was not evident.

The otoliths of the bastard grunt have a ring pattern common to teleost fishes. Marginal increment analysis demonstrated that one annulus, consisting of one opaque zone and one hyaline zone, is formed annually. These rings are believed to be deposited during periods of fast and slow growth, respectively (Williams and Bedford, 1974). Seasonal growth cycles might be related to physiological changes produced by the influence of temperature, feeding regime, and reproductive cycle (Morales-Nin and Ralston, 1990). The seasonalized von Bertalanffy growth model reveals the reduc-

Table 1

Sample size per age group (age-length key) and percentage (in parentheses) within each age group for all fish of *P. incisus* collected off the Canary Islands. n is the number fish by age class and SD is the standar deviation. Total length classes are given in 10-mm intervals.

	Age groups (year)							
Size (mm)	0	I	II	III	IV	V	VI	VII
100	2 (40.0)	3 (60.0)						
110	1 (8.1)	10 (90.9)						
120	1 (5.6)	17 (94.4)						
130		19 (100.0)						
140		15 (71.4)	6 (28.6)					
150		4 (17.4)	19 (82.6)					
160			33 (100.0)					
170			47 (95.9)	2 (4.1)				
180			69 (70.4)	29 (29.6)				
190			52 (46.6)	61 (53.4)				
200			25 (20.5)	96 (78.6)	1 (0.9)			
210			14 (12.8)	89 (81.6)	6 (5.5)			
220			5 (5.9)	57 (67.0)	23 (27.1)			
230				14 (27.0)	38 (73.0)			
240				2 (10.0)	17 (85.0)	1 (5)		
250				1 (6.6)	9 (60.0)	4 (26.8)	1 (6.6)	
260					2 (20.0)	6 (60.0)	2 (20.0)	
270					1 (25.0)	1 (25.0)	2 (50.0)	
280						1 (33.3)	1 (33.3)	1 (33.3)
290							1 (100)	
300								1 (100.0)
Mean	107	125	176	212	236	261	275	295
n	4	68	270	351	97	13	7	2
SD	5.77	12.64	17.28	18.06	12.36	11.43	11.40	14.14

Table 2

Parameters estimates, standard errors, and 95% cofidence intervals for the specialized von Bertalanffy, Schnute, and seasonalized von Bertalanffy growth models for all *P. incisus* collected off the Canary Islands. All models were pooled without the age-0 class.

	95% confidence intervals			
Parameter	Estimate	Standard error	Lower	Upper
Specialized von Bertalanffy growth model (r^2=0.91)				
L_∞ (mm)	309.58	8.06	294.07	325.08
k (/year)	0.220	0.031	0.157	0.283
t_0 (year)	−1.865	0.055	−2.007	−1.723
Schnute growth model (r^2=0.87)				
y_1 (mm)	126.66	1.51	124.54	128.78
y_2 (mm)	293.50	9.50	286.68	300.32
A	−0.426	0.077	−0.578	−0.274
B	5.963	0.664	4.659	7.267
Seasonalized von Bertalanffy growth model (r^2=0.91)				
L_∞ (mm)	309.93	7.68	295.21	324.65
K (/year)	0.218	0.032	0.153	0.282
t_0(year)	−1.896	0.049	−2.067	−1.725
C	0.555	0.212	0.138	0.971
t_s	0.652	0.061	0.532	0.773

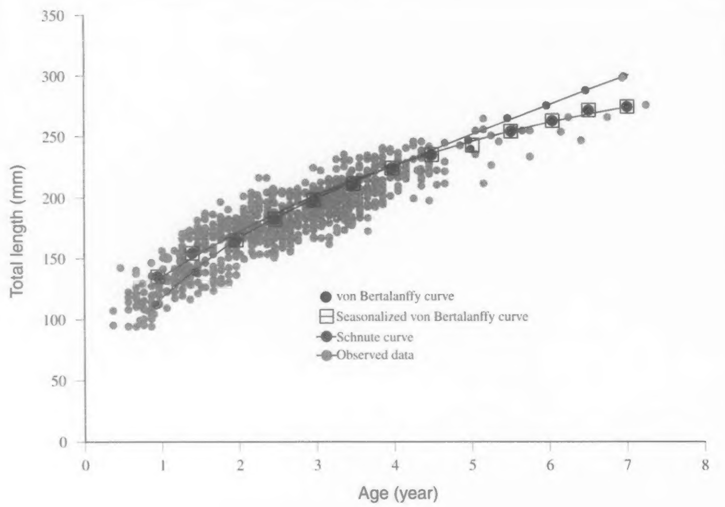


Figure 3

Von Bertalanffy specialized and seasonalized growth curves and the Schnute growth curve for all individuals of *P. incisus* collected off the Canary Islands.

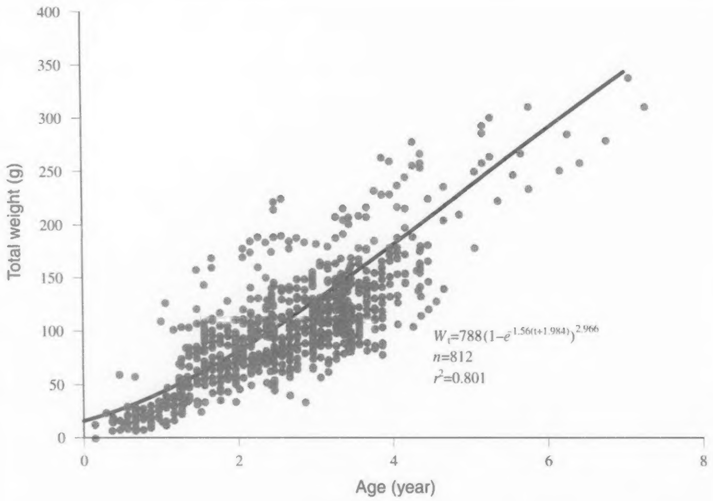


Figure 4

Von Bertalanffy growth curve derived from observed weight at age for all *P. incisus* collected off the Canary Islands.

tion of somatic growth and the formation of the hyaline zone during the winter months. The high correlation found between L_t and otolith radius indicates that otoliths are a useful structure for estimating the age and for indicating the past growth history of bastard grunt.

The coefficients of determination for each fitted curve show that the three models explain more than 88% of the growth pattern. The use of the von Bertalanffy model to describe growth has been criticized for several reasons (Booth, 1997). These include the use of parameters that

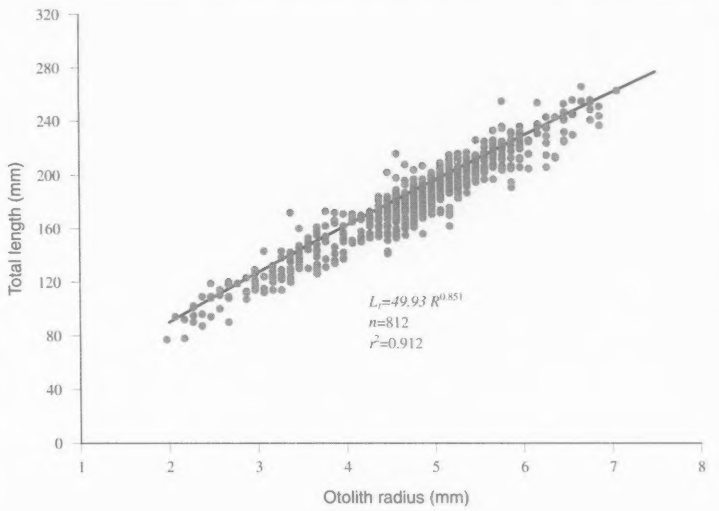


Figure 5

The relation of otolith length to total length for *P. incisus* collected off the Canary Islands.

Table 3

Backcalculated length-at-age for all *P. incisus* collected off the Canary Islands.

Age (year)	Number of fish	Annulus number						
		I	II	III	IV	V	VI	VII
1	68	119						
2	270	123	179					
3	351	127	172	219				
4	97	132	168	212	235			
5	13	129	178	205	245	26		
6	7	122	170	221	233	257	275	
7	2	128	174	214	239	260	279	293
Mean		126	173	214	238	259	277	293
Number of backcalculated lengths at age		808	740	470	119	22	9	2
Annual growth increment (mm)		126	47	41	24	21	18	16
Annual growth increment (%)		43.0	16.1	13.9	8.2	7.2	6.1	5.4

have little biological meaning (Schnute, 1981) and the absence of parameters that take into account seasonal changes in growth rate (Pauly, 1980; Moreau, 1987). Nevertheless, the von Bertalanffy growth model has been used extensively to describe the growth of grunts. The growth model provides a simple description of growth which can be compared between species and species groups (Booth, 1997). The special and the seasonal forms of the von Bertalanffy growth model were chosen for the present study because they contain fewer parameters than the Schnute growth model.

Backcalculated lengths-at-age are in close agreement with the length estimated from otoliths readings. The results obtained with the backcalculation method are very satisfactory because they show the consistency in the interpretation of the sequence of growth increments of the bastard grunt off the Canary Islands and reduce the effect of size-selective sampling bias on the length estimates for youngest fish in the sample (Campana, 2001).

The growth parameters obtained are reasonable because the theoretical maximal length value is higher than the size of the largest fish sampled and the growth coefficient

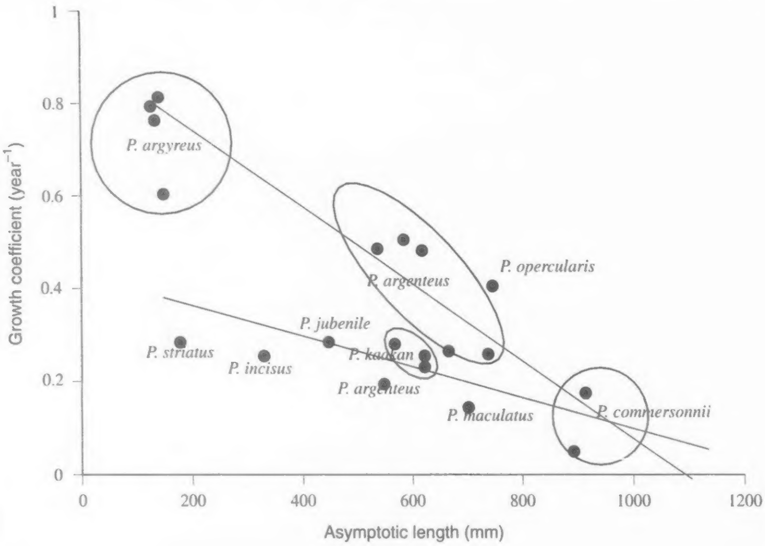


Figure 6

The relation of asymptotic length to the growth coefficient for different species of the *Pomadasys* genus.

value indicates a relatively fast attainment of maximum size, characteristic of the moderately short life cycle for this species. However, the estimations of t_0 tend to be negative and different from zero for values affected by the small sample size of smaller fish. These estimations suggest that the von Bertalanffy growth model does not accurately describe growth in the early stages. *Pomadasys incisus* grows quickly in its first year, attaining approximately 60% of its maximum length. After the first year, the annual growth rate drops rapidly. This change in growth rate is attributable to the utilization of available energy for reproduction instead of somatic growth; in the study area the maturation process begins in the second year of life (Gregoire[1]).

Two different patterns of growth rate in relation to asymptotic length are observed for *Pomadasys* species (Fig. 6). The pattern of *P. incisus* is similar to that observed for *P. striatus, P. jubeline, P. kaakan, P. maculates,* and *P. commersonnii*—species characterized by a high or moderate asymptotic length and low or moderate growth coefficient (Latif and Shenouda, 1972; Wallace and Schleyer, 1979; Edwards et al., 1985; Iqbal, 1989; Al-Husaini et al., 2001; Pauly[2]). However, it differs substantially from that observed for *P. argyreus*, a species with a very low asymptotic length (<151 mm) and a very high growth coefficient (0.62–0.83/year), and for *P. opercularis* and *P. argenteus,* species characterized by a high asymptotic length and a high growth coefficient (550–741 mm; 0.28–0.52/years) (Deshmukh, 1973; Nzioka, 1982; Brothers and Mathews, 1987; Majid and Imad, 1991; Ingles and Pauly[3]).

Results of models used in fisheries management, e.g. analytical yield-per-recruit models (Beverton and Holt, 1957), are sensitive to uncertainty in the estimates of input parameters such as the von Bertalanffy growth parameters. Several estimations of growth in *Pomadasys* species have been derived through length-based methods, which for slow growing species are uncertain. The growth parameters from this study are the first otoliths-based estimates of growth for *P. incisus*. Similar estimates obtained from different growth models and methods suggest that the current estimation could be considered a good estimation of the growth pattern for the species and adequate for use as an input parameter in models for the management of the species.

Acknowledgments

The authors are grateful to the three anonymous reviewers for their constructive, critical, and useful comments on the manuscript.

---

[2] Pauly, D. 1978. A preliminary compilation of fish length growth parameters. Ber. Inst. Meereskunden, Universität an der Kiel 55, 200 p. Institut für Meereskunde, Düsternbrooker Weg 20, 24105 Kiel, Germany.

[3] Ingles, J., and D. Pauly. 1984. An atlas of the growth, mortality and recruitment of Philippine fishes. ICLARM Technical Repport 13, 127 p. World Fish Center (ICLARM) Jalan Batu Maung, Batu Maung, 11960 Bayan Lepas, Penang, Malaysia.

Literature cited

Al-Husaini, M., S. Al-Ayoub, and J. Dashti.
2001. Age validation of nagroor, *Pomadasys kaakan* (Cuvier, 1830) (family: Haemulidae) in Kuwaiti waters. Fish. Res. 53:71–81.

Bauchot, M. L., and J. C. Hureau.
1990. Haemulidae. *In* Check-list of the fishes of the eastern tropical Atlantic (J. C. Quero, J. C. Hureau, C. Karrer, A. Post, and L. Saldanha, eds.), p. 786–787. UNESCO, Paris, France.

Beamish, R. J., and D. A. Fournier.
1981. A method for comparing the precision of a set of age determinations. Can. J. Fish Aquat. Sci. 38:982–983.

Beverton, R. J. H., and S. J. Holt.
1957. On the dynamics of exploited fish populations. Fisheries Investigations Series II, XIX, 533 p. Her Majesty's Stationery Office, London, UK.

Booth, A. J.
1997. On the life history of the lesser gurnard (Scorpaeniformes: Triglidae) inhabiting the Agulhas Bank, South Africa. J. Fish. Biol. 51:1155–1173.

Brothers, E. B., and C. P. Mathews.
1987. Application of otolith microstructural studies to age determinations of some commercially valuable fish of the Arabia Gulf. Kuwait Bull. Mar. Sci. 9:127–157.

Buxton, C. D., and J. R. Clarke.
1991. The biology of the white musselcracker *Sparadon durbanensis* (Pisces: Sparidae) on the Eastern Cape Coast, South Africa. S. Afr. J. Mar. Sci. 10:285–296.

Campana, S. E.
1990. How reliable are growth back-calculations based on otoliths? Can. J. Fish. Aquat. Sci. 47:2219–2227.
2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish. Biol. 59:197–242.

Chang, W. B.
1982. A statistical method for evaluating the reproducibility of age determinations. Can. J. Fish. Aquat. Sci. 39: 1208–1210.

Deshmukh, V. M.
1973. Fishery and biology of *Pomadasys hasta* (Bloch). Indian J. Fish. 20:497–522.

Edwards, R. R. C., A. Bakhader, and S. Shaher.
1985. Growth, mortality, age composition and fishery yields of fish from the Gulf of Aden. J. Fish. Biol. 27:13–21.

Francis, R. I. C. C.
1990. Back-calculation of fish length: a critical review. J. Fish Biol. 36:883–902.

Gayanilo, F. C., P. Sparre, and D. Pauly.
1996. FAO-ICLARM stock assessment tools (FiSAT) user´s guide, 126 p. FAO (Food Agric. Organ. U. N.) Comp. Inf. Ser. (Fish.) 8.

Gordoa, A., and B. Molí.
1997. Age and growth of the sparids *Diplodus vulgaris*, *D. sargus* and *D. annularis* in adult populations and the differences in their juvenile growth patterns in the north-west Mediterranean Sea. Fish. Res. 33:123–129.

Hyndes, G. A, and I. C. Potter.
1997. Age, growth and reproduction of *Sillago schomburgkii* in south-western Australin, nearshore waters and comparisions of life history styles of a suite of *Sillago* species. Environ. Biol. Fish. 49 435–447.

Iqbal, M.
1989. A note on the population dynamics of *Pomadasys kaakan* (Haemulidae) from Pakistan. Fishbyte 7:4–5.

Latif, A., and S. Shenouda.
1972. Biological studies on *Rhonciscus striatus* (family Pomadasidae) from the Gulf of Suez. Bull. Inst. Ocean. Fish. 2:103–134.

Majid, A., and A. Imad
1991. Growth of *Pomadasys kaakan* (Haemulidae) off the coast of Pakistan. Fishbyte 9:30–33.

Morales-Nin, B., and S. Ralston.
1990. Age and growth of *Lutjanus kasmira* (Forskal) [*sic*] in Hawaiian waters. J. Fish Biol. 36:191–203.

Moreau, J.
1987. Mathematical and biological expression of growth in fishes: recent trends and further developments. *In* Age and growth of fish (R. C. Summerfelt and G. E. Hall, eds.), p. 81–113. Iowa State Univ. Press, Ames, IA.

Nzioka, R. M.
1982. Biology of the small spotted grunt *Pomadasys opercularis* (Playfair 1866) (Pisces: Pomadasyidae) around Malindi in Kenya. Kenya J. Sci. Tech. 3:69–81.

Pilling, G. M., R. S. Millner, M. W. Easey, C. C. Mees, S. Rathachasen, and R. Azemia.
2000. Validation of annual growth increments in the otoliths of the lethrinid *Lethrinus mahsena* and the lutjanid *Aprion virescens* from sites in the tropical Indian Ocean, with notes on the nature of growth increments in *Pristipomoides filamentosus*. Fish. Bull. 98:600–611.

Pauly, D.
1980. On the interrelationships between natural mortality, growth parameters, and mean enviromental temperature in 175 fish stocks. J. Cons. Int. Explor. Mer 39:175–195.

Pitcher, T. J., and P. D. M. Macdonald.
1973. Two models for seasonal growth in fishes. J. Appl. Ecol. 10:597–606.

Ricker, W. E.
1973. Linear regressions in fishery research. J. Fish. Res. Board Can. 30:409–434.

Schnute, J.
1981. A versalite growth model with statistically stable parameters. Can. J. Fish. Aquat. Sci. 38:1128–1140.

Sparre, P., and S. C. Venema.
1995. Introducción a la evaluación de recursos pesqueros tropicales, parte 1, manual, 420 p. FAO (Food Agric. Organ. U. N.) Doc. Téc. Pesca 306(1).

Wallace, J. H., and M. H. Schleyer.
1979. Age determination in two important species of South Africa angling fishes, the knob (*Argyrosomus hololepidotus* Lacep) and the spotted grunter (*Pomadasys commersonnii* Lacep). Trans. R. Soc. S. Afr. 44:15–26.

Williams, T., and B. C. Bedford.
1974. The use of otoliths for age determination. *In* The ageing of fish (T. B. Bagenal, ed.), p. 114–123. Unwin Brothers, Old Woking, Surrey, UK.

Evaluating the efficacy of managing West Coast groundfish resources through simulations

André E. Punt
School of Aquatic and Fishery Sciences
University of Washington
1122 NE Boat Street
Seattle, Washington 98195-5020
E-mail address: aepunt@u.washington.edu

Abstract—Management of West Coast groundfish resources by the Pacific Fishery Management Council involves Federal government and academic scientists conducting stock assessments, generally using the stock synthesis framework, applying the 40-10 rule to determine harvest guidelines for resources that are not overfished and conducting rebuilding analyses to determine harvest guidelines for resources that have been designated as overfished. However, this management system has not been evaluated in terms of its ability to satisfy the National Standard 1 goals of the Sustainable Fisheries Act. A Monte Carlo simulation framework is therefore outlined that can be used to make such evaluations. Based on simulations tailored to a situation similar to that of managing the widow rockfish (*Sebastes entomelas*) resource, it is shown that catches during recovery and thereafter are likely to be highly variable (up to ±30% from one year to the next). Such variability is far greater than has been presented to the decision makers to date. Reductions in interannual variability in catches through additional data collection are, however, unlikely. Rather, improved performance will probably arise from better methods for predicting future recruitment. Rebuilding analyses include quantities such as the year to which the desired probability of recovery applies. The estimates of such quantities are, however, very poorly determined.

Manuscript approved for publication
24 April 2003 by Scientific Editor.
Manuscript received 26 June 2003
at NMFS Scientific Publications Office.
Fish. Bull. 101:860–873 (2003).

National Standard 1 of the Sustainable Fisheries Act (SFA) of 1996 states that "Conservation and management measures shall prevent overfishing while achieving, on a continuing basis, the optimum yield from each fishery for the United States industry." The need to satisfy this National Standard has led *inter alia* to the requirement for the eight Regional Fishery Management Councils to develop control rules that are used to assess whether overfishing is occurring[1] or a stock is in an overfished state (e.g. Restrepo and Powers, 1999). In addition, the SFA specifies that a rebuilding plan has to be developed for any fish stocks that are designated as overfished. This plan needs to include the time period by which the stock will be rebuilt to B_{MSY} (the average biomass associated with maximum sustainable yield, MSY), and the strategy by which the stock is to be rebuilt.

The Pacific Fishery Management Council (PFMC) has adopted the "40-10" rule to manage groundfish stocks that are not designated as being overfished. This rule determines the harvest guideline for each groundfish stock by computing the catch corresponding to an F_{MSY} proxy ($F_{40\%}$[2] for flatfish, $F_{50\%}$ for rockfish in the *Sebastes* complex, and $F_{45\%}$ for other species) and reducing it if the spawning output is estimated to be less than 40% of the estimated B_0. This reduction in catch is linear with spawning output, being 0 at $0.4B_0$ and 100% at $0.1B_0$. For stocks that are designated as being in an overfished state (defined for West Coast groundfish as being that the spawning output is less than $0.25B_0$) a rebuilding plan is developed.[3] The main features of the technical aspects of a rebuilding plan (referred to as a rebuilding analysis) identified by the Scientific and Statistical Committee of the PFMC are outlined in Appendix 1. In brief, the rebuilding analysis used by the PFMC involves projecting the best estimates of the current age-structure of the overfished population forward under a range of alternative fishing mortality rates and selecting the fishing mortality rate that has a Council-selected probability that the population recovers to the proxy for B_{MSY} of $0.4B_0$ within a time frame consistent with the specifications of the SFA.

Detailed stock assessments are available for only a small subset of the 81 species included in the PFMC Groundfish Management Plan. Of these species, nine (bocaccio [*Sebastes paucispinis*], canary rockfish [*Sebastes pinniger*], cowcod [*Sebastes levis*], darkblotched rockfish [*Sebastes crameri*], lingcod [*Ophiodon elongates*], Pacific ocean perch [*Sebastes alutus*], Pacific whiting [*Merluccius productus*], widow rockfish [*Sebastes entomelas*], and yelloweye rockfish [*Sebastes ruberrimus*]) have been designated overfished and rebuilding plans have been or are being developed for them. The direct consequences for industry of the implementation of a rebuilding plan can be substantial (e.g. a reduction in the catch of canary rockfish from 883 metric tons (t) in 1999 to only 90 t in 2001), although there are also indirect consequences in the form of reductions in the harvest of nonoverfished species to prevent overharvesting of overfished species through technical interactions.

---

[1] In the present study, and consistent with usage by the Pacific Fishery Management Council, "overfishing" means that the level of fishing mortality exceeds that associated with MSY and "being in an overfished state" means that the current spawning output is less than 25% of the pre-exploitation equilibrium spawning output, B_0 (spawning output is the product of egg production-at-age and numbers-at-age).

[2] $F_{x\%}$ is the fishing mortality rate at which the spawning output-per-recruit is reduced to x% of its unfished level.

[3] One implication of this is that the 40-10 rule is not actually used if the stock is assessed to be below $0.25B_0$.

The performance of the method commonly used for assessments of West Coast species has been evaluated to some extent (e.g. Sampson and Yin, 1998; Ianelli, 2002). However, the performance of this assessment method in combination with the rules used to determine harvest guidelines has not been evaluated.

Management procedures[4] are combinations of stock assessment methods and catch control laws that have been evaluated by means of Monte Carlo simulation to assess the extent to which they are able to satisfy the management objectives for a fishery. Evaluation of management procedures by means of Monte Carlo simulation has been argued to be essential because "if a management procedure is unable to perform adequately in the ideal world represented on a computer, what basis is there to assume that it will perform adequately in the real world?" (Sainsbury[5]). One caveat to this argument is that it is only possible to evaluate a management procedure if it is fully specified and if it will be followed for several years in reality.

Management procedures have been adopted by the International Whaling Commission for managing commercial and aboriginal whaling (e.g. IWC, 1992, 2001) and by southern African nations for managing a variety of pelagic and demersal resources (Butterworth and Bergh, 1993; Cochrane et al., 1998; Geromont et al., 1999). Management procedures are under consideration in Australia (Punt et al., 2001) and New Zealand (Starr et al., 1997). If it can be assumed that the same rules will be applied to modify rebuilding plans each time new information on abundance and year-class strength becomes available, it is possible to consider the combination of the assessment method, the default 40-10 rule, and rebuilding plans as a "management procedure" and evaluate it by means of Monte Carlo simulation. This study therefore involves determining from past practice the "management procedure" being applied by the PFMC. However, this "management procedure" has not been formally adopted in any way and the approach to managing West Coast groundfish could change in time.

This paper first outlines a simulation framework (a management procedure evaluation, MPE, framework) within which the expected performance of the approach used by the PFMC to determine harvest guidelines can be evaluated. It then evaluates variants of this approach for scenarios similar to that of managing the fishery for widow rockfish.

Materials and methods

The steps in evaluating management procedures are as follows:

1. Identification of the management objectives and representation of these by using a set of quantitative performance statistics.
2. Identification of a range of alternative management procedures.
3. Development and parameterization of a set of alternative structural models (called operating models) of the system.
4. Simulation of the future use of each management procedure to manage the system (as represented by each operating model). For each year of the projection period, the simulations involve the following steps:
    a. Generation of the data available for assessment purposes.
    b. Application of a method of stock assessment to the generated data to determine key assessment-related quantities (e.g. current age-structure, spawning output in relation to target and limit levels, historical trends in recruitment) and any inputs to the catch control law.
    c. Application of the catch control law element of the management procedure to determine a harvest guideline.
    d. Determination of the biological implications of this harvest guideline by setting the catch for the "true" population represented in the operating model based on it. The step can potentially include "implementation uncertainty" (Rosenberg and Brault, 1993).

The harvest guideline is not updated every year in the simulations described in this article, but rather every third year (co-incident with the results from each new survey) and thus reflects the intended frequency with which assessments for West Coast groundfish species are conducted. Each simulation trial (i.e. each combination of an operating model variant and candidate management procedure) involves 100 simulations of an 80-year management period. The four steps listed above are discussed in detail below.

Note that for the application considered in this paper then, there are three "models": 1) the operating model that represents "reality" for the simulations, 2) an assessment model (a stock synthesis-like approach), and 3) a model to calculate the harvest guidelines. The data available to the last two models are generated from the first model.

The operating model

The operating model has been taken to be virtually identical to that on which the population assessments and rebuilding analysis calculations are based (Appendix 1), with two exceptions: 1) the approach used to generate recruitment and 2) the allowance for variability over time in commercial selectivity. Commercial selectivity is given

---

[4] Also referred to as "harvest strategies" (Punt et al., 2001), "management decision rules" (Starr et al., 1997), "fisheries control systems" (Hilborn, 1979), and "operational management procedures" (Barnes, 1999).

[5] Sainsbury, K. G. 2001. Personal commun. CSIRO Marine Research, Castray Esplanade, Hobart, TAS 7000, Australia.

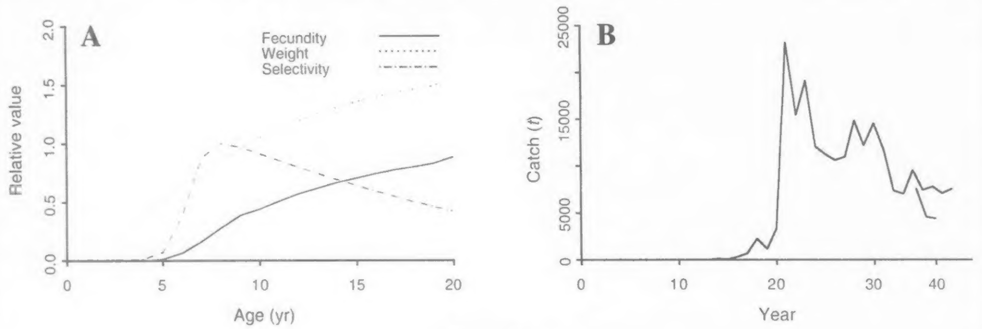


Figure 1

Biological parameters (**A**) and catch history (**B**) for widow rockfish (*Sebastes entomelas*).

by the following double-logistic equation:

$$S_{y,a} = S'_{y,a} / \max_{a'} S'_{y,a'}$$
$$S'_{y,a} = \frac{1}{1+e^{-\delta_1(a-a^1_{50}+\gamma_y)}}\frac{1}{1+e^{-\delta(a^2_{50}-a)}}, \qquad (1)$$

where $S_{y,a}$ = the selectivity on fish of age a during year y;

$a^1_{50}, a^2_{50}, \delta_1, \delta_2$ = the parameters of the double-logistic equation;

γ_y = the deviation from the average selectivity pattern in year y:

$$\gamma_y = \rho_s\, \gamma_{y-1} + \varepsilon^S_y \qquad \varepsilon^S_y \sim N(0;\sigma^2_S),$$

ρ_S = the interannual correlation in the deviation from average selectivity; and

σ_S = a measure of the standard deviation of the interannual deviations from average selectivity.

Recruitment is assumed to be governed by a Beverton-Holt stock-recruitment relationship:

$$R_y = \frac{R_0 4h(\tilde{B}_y / B_0)}{4h+(5h-1)(\tilde{B}_y / B_0 - 1)} e^{\varepsilon^R_y - \sigma^2_R/2} \qquad \varepsilon^R_y \sim N(0;\sigma^2_R), \quad (2)$$

where R_0 = the "virgin recruitment" (the number of zero-year-olds at the pre-exploitation equilibrium level);

$\tilde{B}_y$ = the spawning output at the start of year y;

h = the "steepness" of the stock-recruitment relationship (the fraction of virgin recruitment expected at $0.2B_0$); and

σ_R = the standard deviation of the logarithms of the random fluctuations in recruitment about its expected value.

The biological parameters of the operating model are set to those for widow rockfish (Fig. 1A), and the catches for the 40 years prior to the year in which the management procedure is first applied (referred to as "projection year 1") are set to the actual catches for widow rockfish (Fig. 1B). The baseline values for the parameters h, σ_R, ρ_S, and σ_S (Table 1) are educated guesses. The baseline choice for steepness, h, is lower than the posterior mean for this quantity (0.65) obtained by Dorn (2002) because, increasingly, West Coast rockfish are being found to be less productive than initially anticipated (e.g. Ianelli, 2002). The value assumed for the extent of variation in recruitment, σ_R, although based on the collection of estimates of this parameter by Beddington and Cooke (1983), is nevertheless also largely an educated guess. Sensitivity to the values for both h and σ_R is explored.

Table 1

The baseline parameters of the operating model and the values used in the tests of sensitivity. N/A = not available.

Parameter	Baseline value	Sensitivity values
ρ_S	0.707	N/A
σ_S	0.4	N/A
h	0.4	0.25; 0.7
σ_R	0.6	0.4; 1
M	0.15/yr	N/A
Spawning output in year 41	$0.2B_0$	$0.1B_0$; $0.4B_0$

The biomass at the start of year 1 is assumed equal to B_0, which is defined as the mean of the distribution for the unfished biomass which would arise given variability in recruitment about its expected value. However, this specification has little impact on the results. For example, the alternative that is defined to be the median of the distribution for the unfished biomass would only change B_0 by about 5%.

The value for B_0 for each simulation is selected so that the spawning output at start of year 41 (projection year 1) equals a prespecified fraction of B_0 (baseline fraction

Table 2

The parameters on which the generation of future data is based. n^e is the sample size for the multinomial distribution.

Data source	First year collected	Frequency	Precision
Catch rates	14	Every year	$\sigma^c = 0.4$
Fishery age-composition	21	Every year	$n^e=200$
Survey indices	13	Every third year	$\sigma^s=0.5$
Survey age-composition	13	Every third year	$n^e=200$

0.2—i.e. just below the level that defines an overfished stock). Sensitivity to alternative values for the ratio of the spawning output at the start of year 41 to B_0 is explored (Table 1).

Generating future data

The data available for assessment purposes are survey indices of relative abundance, age-composition data from surveys, catch-rate–based indices of relative abundance, and age-composition data from the commercial catches. Table 2 lists the baseline specifications regarding the frequency at which the various data sources are collected and the parameters that determine the sampling variability associated with each data source.

The survey and catch-rate indices are generated by using the equations

$$B_y^{s,obs} = B_y^s e^{\varepsilon_y^s - (\sigma^s)^2/2}, \qquad \varepsilon_y^S \sim N\left(0;(\sigma^s)^2\right); \qquad (3a)$$

$$I_y = B_y^e e^{\varepsilon_y^c - (\sigma^c)^2/2}, \qquad \varepsilon_y^c \sim N\left(0;(\sigma^c)^2\right); \qquad (3b)$$

where $B_y^{s,obs}$ = the survey index for year y;

B_y^s = the survey selected-biomass during year y:

$$B_y^s = \sum_{a=0}^{a_{\max}} w_a S_a^s N_{y,a} e^{-Z_{y,a}/2}; \qquad (4a)$$

w_a = the mass of an animal of age a;

S_a^s = the selectivity of the survey gear on animals of age a (assumed to be governed by a logistic function and to be independent of time);

$N_{y,a}$ = the number of animals of age a at the start of year y;

$Z_{y,a}$ = the total mortality on animals of age a during year y;

σ_s = the standard deviation of the random fluctuations in survey catchability;

$a_{\max}$ = the oldest age considered in the operating model;

I_y = the catch-rate index for year y;

B_y^e = is the exploitable biomass during year y;

$$B_y^e = \sum_{a=0}^{a_{\max}} w_a \frac{S_{y,a}F_y}{Z_{y,a}} N_{y,a}(1-e^{-Z_{y,a}}); \qquad (4b)$$

F_y = the fully selected fishing mortality during year y; and

σ^c = the standard deviation of the random fluctuations in fishery catchability.

Note that Equations 3a and 3b assume that the survey and fishery catchability coefficients are unity. This assumption can be made without loss of generality because the stock assessment method is not provided with this information and instead estimates these catchability coefficients. Note also that the key difference between the survey index and the catch-rate index is that selectivity for the latter changes over time (see Eq. 1), whereas selectivity for the former is time-invariant.

The age-composition data are generated by selecting a sample multinomially from the age-composition of the survey catch and of the fishery catch (see Eqs. 5a and 5b for the relative survey and fishery catches-at-age):

$$S_a^s n_{y,a} e^{-Z_{y,a}/2}; \qquad (5a)$$

$$\frac{S_{y,a}}{Z_{y,a}} n_{y,a}(1-e^{-Z_{y,a}}). \qquad (5b)$$

The PFMC management procedure

The "PFMC management procedure" (see Fig. 2 for an overview) involves first conducting a stock assessment by fitting an age-structured population dynamics model to the available data by maximizing a likelihood function. This approach mimics the common use of the stock synthesis framework (Methot, 2000) when conducting assessments of West Coast groundfish resources. The likelihood function is determined by assuming that the age-composition data are multinomially distributed (in the simulations with effective sample sizes given by the actual effective sample sizes) and by assuming that the survey and catch-rate series are log-normally distributed about the appropriate model quantities. The estimable parameters of the model are the annual recruitments, the annual fishing mortalities, the catchability coefficients, and the parameters that determine selectivity (the survey and fishery selectivity are [correctly] assumed to be governed by logistic and double-logistic equations). The values for the remaining parameters (weight-at-age, fecundity-at-age, and natural mortality) are assumed to be known without error. The key outputs from the assessment are time-series of recruitments and spawning out-

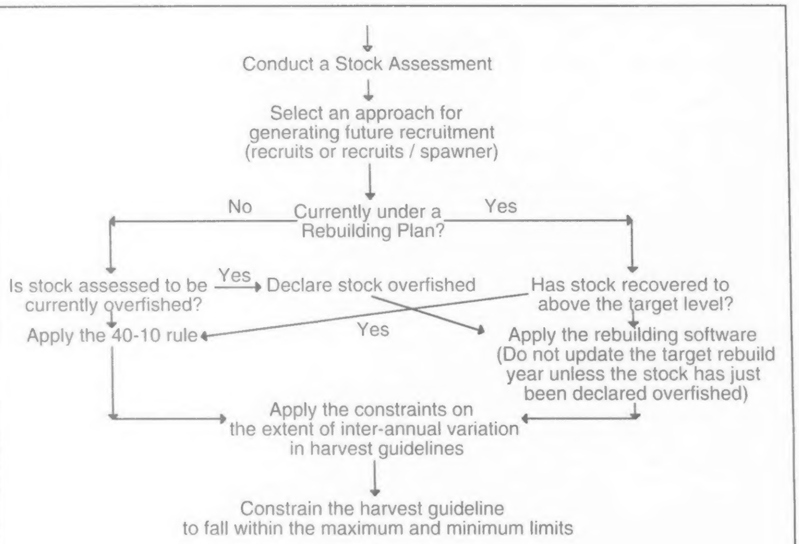

Conduct a Stock Assessment
Select an approach for generating future recruitment (recruits or recruits / spawner)
No
Currently under a Rebuilding Plan?
Yes
Is stock assessed to be currently overfished?
Yes
Declare stock overfished
Has stock recovered to above the target level?
Yes
Apply the 40-10 rule
Apply the rebuilding software (Do not update the target rebuild year unless the stock has just been declared overfished)
Apply the constraints on the extent of inter-annual variation in harvest guidelines
Constrain the harvest guideline to fall within the maximum and minimum limits

Figure 2

Flowchart of the Pacific Fishery Management Council management procedure.

puts, and the age structure at the start of the last year of the assessment.

An estimate of the pre-exploitation equilibrium spawning output (i.e. $\hat{B}_0$) is obtained by multiplying the average recruitment for the first ten years of the assessment period by the spawning output-per-recruit in the absence of fishing. This approach to estimating $\hat{B}_0$ has been used for several rebuilding analyses for West Coast groundfish species. If the estimate of the current spawning output exceeds $0.4\hat{B}_0$ or if it exceeds $0.25\ \hat{B}_0$ and the resource is not currently under rebuilding (i.e. has not yet been declared to be in an overfished state), a raw harvest guideline is computed using the 40-10 rule. On the other hand, if the estimate of the current spawning output is less than $0.25\ \hat{B}_0$ or the stock is currently under a rebuilding plan and the spawning output has not yet recovered to $0.4\ \hat{B}_0$, the raw harvest guideline is based on the application of the rebuilding analysis (see Appendix 1 for further details).

It is necessary to know the maximum possible rebuilding period, $T_{\max}$, when using a rebuilding analysis to calculate a harvest guideline. If the stock is declared overfished in the present year, $T_{\max}$ is computed as described in Appendix 1. On the other hand, if the stock is currently under a rebuilding plan, $T_{\max}$ is taken to be the value computed when the stock was first declared overfished. Therefore, the implementations of the rebuilding plans considered in this paper are based on the assumption that the $T_{\max}$ and the probability of recovery by $T_{\max}$ are set when the first rebuilding analysis is conducted and not changed thereafter. The probability of recovery by $T_{\max}$ is taken to be 0.6 in this paper because this is the probability on which management of widow rockfish is currently based. This probability ranges between 0.55 and 0.92 among the seven overfished groundfish resources for which it has been selected by the PFMC.

Calculation of a harvest guideline using the 40-10 rule and application of the rebuilding analysis requires the ability to generate future recruitment. For the purposes of the present study (and consistent with current practice), future recruitment is either generated from the estimates of recruitment from the assessment or by multiplying the spawning output by a generated value for the recruits-per-spawning output ratio. The pool of recruitment to recruits-per-spawning output is taken to be those for the last 23 years of the assessment period less those for the last three years. The last three years are excluded because of their known poor precision. The approach used to generate recruitment therefore leads to the set of recruitments used to conduct projections changing with time. Allowing the set of recruitments to change with time is needed to avoid an inconsistency between the recruitments used for projections and the recruitments on which the estimate of B_0 is based.

Allowance is made for the raw harvest guideline to be constrained so as not to change by more than a prespecified percentage from that for the previous year and not to fall outside of specified limits, although this option is not part of the baseline simulations.

One aspect of the actual management process that is ignored in the simulation of the PFMC management procedure is the time-lag between the collection of data and their use in assessments (for example, catch-at-age information from surveys conducted in one year would usually not be available for use in the assessments conducted in the following year) and that between assessments

Table 3

The performance statistics used in the present study. For consistency with the definition of recovery used by the Pacific Fishery Management Council, "recovery by year x" is defined as the spawning output being larger than $0.4B_0$ at or before year x. Some of the statistics are based on the "actual" (i.e. operating model) spawning output and others are based on the "assessed" (i.e. assessment model) spawning output.

Abbreviation	Description
F_{rec}	The fraction of the simulations in which the stock is assessed to be overfished at the start of the first projection year that actually recover by the maximum possible recovery year determined from the rebuilding analysis conducted in projection year 1.
Y_{rec}	The median year in which the actual spawning output first reaches $0.4B_0$.
P_{decl}	The proportion of simulations in which the spawning output is assessed to be below $0.25B_0$ (i.e. overfished) at the start of projection year 1.
$5\%D/50\%D$	The lower 5th and median of the distribution of the actual spawning output in projection years 20 and 60 expressed in relation to the actual pre-exploitation spawning output, B_0.
AAV	Average annual absolute change in catch evaluated after 20 and 60 years, i.e. $AAV = \sum_y \lvert C_y - C_{y-1} \rvert / \sum_y C_y$, where C_y is the catch during year y.
$\overline{C}$	Average annual catch over projection years 1–20 and 1–60.
P_{rec}	The fraction of simulations in which actual spawning output reached $0.4B_0$ sometime between projections years 1 and 20 and between projection years 1 and 60 (but may have dropped below $0.4B_0$ again).

being conducted and their being used for management purposes.

The performance statistics

A variety of performance statistics are considered (Table 3). These consider both the performance of the management procedure in terms of the behavior of the rules used for management (statistics F_{rec}, Y_{rec}, and P_{decl}) and of satisfying the goals established by the SFA in relation to the status of the population and the fishery (statistics $5\%D$, $50\%D$, $\overline{C}$, AAV, and P_{rec}). The choice of years 20 and 60 in the definitions of the latter five statistics is meant to capture "short"-term and medium-term considerations. For instance, recovery should have occurred by year 60 in most cases and the population should be well above $0.25B_0$ after 20 years. The catch and catch variability statistics for the first 20 years provide an indication of the likely impacts of recovery on the industry.

The need to examine aspects of the behavior of the management rules can be understood from Figure 3, which shows results for four simulations for the combination of a PFMC management procedure and an operating model variant. The solid lines are the "true" time-trajectories of spawning output (expressed in relation to the pre-exploitation level) and the dotted lines reflect the estimates of this ratio each time an assessment is conducted (every third year for the analyses shown in Fig. 3). The up arrows indicate when the assessment first indicates that the population is overfished (based on the model estimates of spawning output)—note that a population may be identified to be overfished more than once during a given simulation. The down arrows indicate the years in which recovery is predicted by the rebuilding analysis software (with the estimates from the assessment) to occur with 60% probability. The solid bar parallel to the x-axis indicates the years in which management is based on the rebuilding plan (rather than the 40-10 rule). The bar will stretch from the up arrow to the down arrow unless the population is assessed to have recovered to $0.4B_0$ (when management reverts to being based on the 40-10 rule).

There are several possible impacts of the difference between the perceived and true state of the system. For example, the population can erroneously be assessed not to be overfished in the first projection year (e.g. simulation 1 in Fig. 3). The statistic P_{decl} is designed to capture the frequency of this possibility. Even if the population is assessed to be overfished, there is no guarantee that it will recover with the expected probability and in the "correct" year. For example, for simulation 1, the stock assessment indicates that recovery occurs in year 71 (the solid bar consequently stops in year 71) even though the true population size is less than 30% of B_0 at that time. The statistic F_{rec} attempts to capture whether the rebuilding analysis performs as expected given that the population is assessed to be overfished at the start of the first projection year.

There are other aspects to evaluating the behavior of the management rules in relation to the perceived and true state of the system (e.g. the difference between the true and estimated biomasses and recruitments). Although it is straightforward to evaluate these aspects (e.g. Patterson and Kirkwood, 1995; Punt et al., 2002), they are not considered in detail in this paper to reduce the volume of results presented.

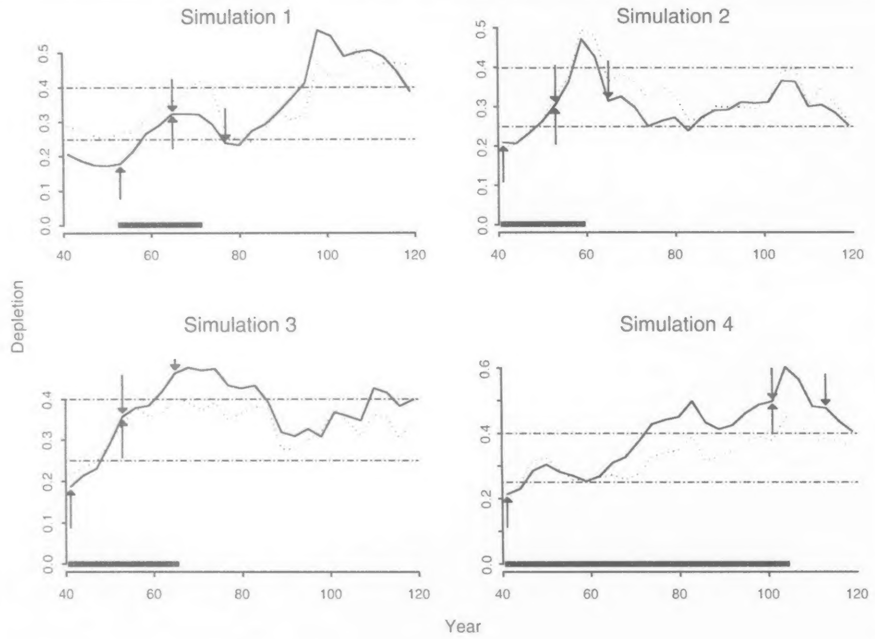


Figure 3

Time-trajectories of the "true" and the assessment model-estimated ratio of the spawning output to B_0 (depletion) for four simulations. The up arrows indicate the years in which the stock was declared to be in need of rebuilding and the down arrows show the values of T_{max}. The horizontal bars indicate the years during which the stock is under a rebuilding plan. Year 41 is the first "projection year," i.e. the first year in which the management procedure is used to determine the catches (the catches for the years prior to year 41 are set equal to the historical catches—see Fig. 1A)

Results and discussion

Detailed results for a single operating model variant and management procedure

Figures 4 and 5 and Table 4 summarize aspects of a simulation trial in which the operating model has its baseline parameterization (Tables 1 and 2) and in which the management procedure used to set harvest guidelines is the PFMC management procedure with no constraints on interannual variation in harvest guidelines other than an upper limit of 10,000 t. The lack of any constraints on changes in harvest guidelines has been imposed because the PFMC has not adopted any such constraints. The harvest guideline is updated every third year.

Figure 4 shows the time-trajectories of catch, spawning output in relation to the pre-exploitation equilibrium level ("true" and estimated), and the perceived fishing mortality on which the harvest guideline is based for three of the 100 simulations that constitute a simulation trial. The horizontal bars on the x-axis again reflect the year during which the stock is managed by using the results from the rebuilding analysis rather than the 40-10 rule. The most notable feature of Figure 4 is the high variability in annual catches. This variability arises for several reasons: 1) the additional information on population biomass obtained each time a survey occurs changes the perceived status of the resource and hence how far the spawning output is from the target level of $0.4B_0$; 2) an extension of the assessment period changes the set of recruitments on which generation of future recruitment is based; and 3) a change from being under a rebuilding plan to being managed by means of the 40-10 rule can lead to marked changes in catch. The latter is evident by the change in fishing mortality and catch when the spawning output is estimated to reach $0.4B_0$ (i.e. the end of the horizontal bar). A marked impact due to the addition of data for a single 3-year period may appear surprising. However, effects of this nature have already been observed for West Coast species (see, for example, the 2002 update to the sablefish [*Anoplopoma fimbria*] stock assessment [Schirripa and Methot[6]).

---

[6] Schirripa, M. J., and R. Methot. 2002. Status of the sablefish resource off the continental U.S. Pacific Coast in 2001. *In* Stock assessment and fishery evaluation: appendix to the status of the Pacific Coast groundfish fishery through 2001 and acceptable biological catches for 2002, x + 122 p. Pacific Fishery Management Council, 7700 NE Ambassador Place, Portland, OR 97220.

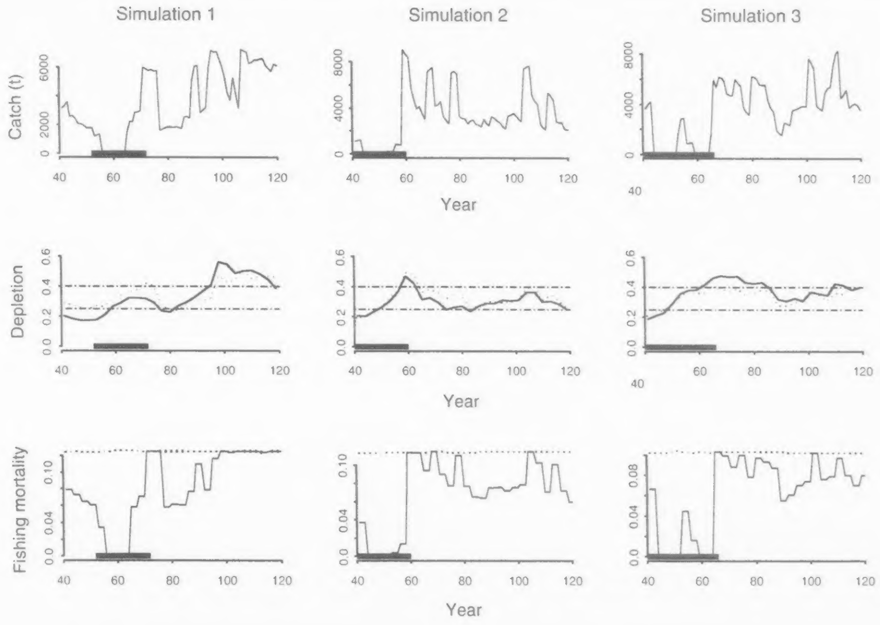


Figure 4

Time-trajectories of catch (**upper panels**), spawning output in relation to the pre-exploitation level (solid line is "true"; dotted line is estimated) (**center panels**), and perceived fishing mortality (used to set the harvest guideline [solid line]; dotted line=F_{MSY} proxy) (**lower panels**) for three individual simulations. The results in this figure pertain to the baseline operating model and baseline Pacific Fishery Management Council management procedure.

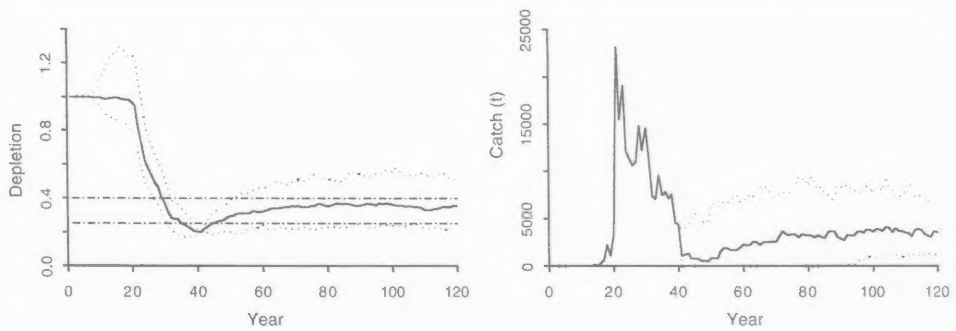


Figure 5

Piecewise medians (solid lines) and 90% intervals (dotted lines) for spawning output in relation to the pre-exploitation equilibrium level (left panel) and catch (right panel). The results in this figure pertain to the baseline operating model and baseline Pacific Fishery Management Council management procedure.

The extent of variability in catch in Figure 4 differs markedly from the way advice on expected catches during the rebuilding period is presented to the decision makers (e.g. Fig. 6). One way to improve the presentation of information on expected catches would be to include some individual catch trajectories from those on which the rebuilding analysis is based. However, even these would severely underestimate the actual extent of uncertainty

Table 4

Performance statistics (see Table 3 for definitions) for six alternative management procedure variants. All of the calculations in this table relate to the baseline operating model. PFMC = Pacific Fishery Management Council. N/A = not applicable.

				Results after 20 years					Results after 60 years				
Management procedure	F_{rec}	Y_{rec}	P_{decl}	5%D	50%D	AAV	$\overline{C}$	P_{rec}	5%D	50%D	AAV	$\overline{C}$	P_{rec}
Baseline	0.22	72	0.82	0.22	0.33	0.33	1759	0.32	0.23	0.36	0.25	2847	0.80
With constraints	0.27	61	0.82	0.24	0.40	0.38	591	0.54	0.24	0.41	0.17	2440	0.89
No 10 years and estimated F_{MSY}	0.42	68	0.82	0.24	0.34	0.30	1652	0.27	0.25	0.41	0.24	2649	0.84
Preferred	0.59	62	0.82	0.25	0.39	0.31	950	0.49	0.28	0.54	0.21	1961	0.96
PFMC (baseline)	N/A	95	N/A	0.19	0.29	0.23	2273	0.07	0.24	0.33	0.20	2851	0.55
PFMC (preferred)	N/A	64	N/A	0.23	0.36	0.30	1239	0.45	0.30	0.48	0.20	2265	0.93

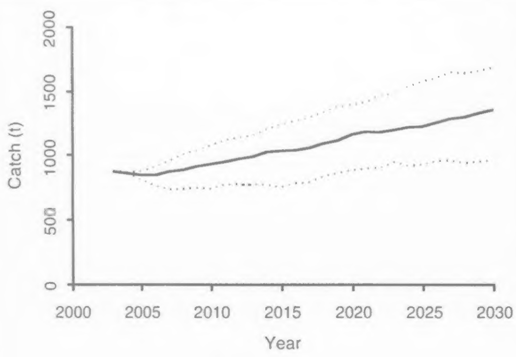


Figure 6

Time-trajectories of catch (median and 95% intervals) for the annual catch for widow rockfish based on a rebuilding analysis conducted in 2002.

because they are conditioned on knowing the age-structure of the population at the start of the projection period and are based on fixed levels of fishing mortality during the rebuilding period.

The impact of estimation uncertainty is also evident in Figure 4. The following are three examples of this: 1) management based on the rebuilding plan only starts in year 53 in simulation 1 because, prior to this year, the stock assessment indicates (erroneously) that the stock is above rather than below $0.25B_0$; 2) the resource is predicted to have recovered to $0.4B_0$ in year 71 in simulation 1 (and hence management is based on the 40-10 rule thereafter)—however, the spawning output is really only slightly larger than $0.3B_0$ at this time; and 3) in simulation 3 the assessment model indicates that the spawning output has recovered to above $0.4B_0$ in year 65 when, in fact, it recovered to $0.4B_0$ three years earlier.

The results of all 100 simulations are summarized by the time-trajectories in Figure 5. The trajectories of catch in Figure 5 are notably less variable that the individual trajectories in Figure 4 because, for instance, the 5th, median, and 95th intervals for the catch in year 80 are obtained by sorting all 100 year-80 catches and taking the appropriate percentiles. Unlike the individual trajectories, the median trajectories of catch and spawning output show quite smooth changes over time. This result highlights the importance of the AAV statistic that captures interannual variation in catches within individual simulations.

Overall, there is a high probability (0.82) that the assessment model identifies that the spawning output is less than $0.25B_0$ at the start of the projection period (Table 4). However, the probability that recovery occurs at or before the T_{max} year predicted from the rebuilding analysis conducted in projection year 1 is rather low (0.22) and 50% of simulations exceed $0.4B_0$ only in year 72 (i.e. after 30 years). The probability of being below the overfished level of $0.25B_0$ still exceeds 5% after 60 years of management with this management procedure although there is an 80% probability that the spawning output recovers to $0.4B_0$ sometime during the first 60 years of management with the management procedure.

It should be noted that the impact of recruitment variability and assessment errors following recovery to $0.4B_0$ can be consequential. For example, the probability of having reached $0.4B_0$ after 60 years of management by using the management procedure exceeds 0.8 but the median value of the ratio of the spawning output in year 60 to B_0 is nevertheless still less than 0.4 (Table 4, Fig. 5). One reason for the spawning output not stabilizing at $0.4\,B_0$ is a discrepancy between the fishing mortality rate that stabilizes the population at B_0 (deterministically) and $F_{50\%}$. For the baseline steepness of 0.4, the fishing mortality required to stabilize the spawning output at $0.4\,B_0$ actually corresponds to a lower fishing mortality than $F_{50\%}$ (closer to $F_{63\%}$).

Sensitivity to alternative management procedures

Table 4 includes results for a range of variants of the baseline management procedure designed to improve its performance. The following are areas where improved performance is desirable: 1) the extent of interannual variability in catches; 2) the similarity between the year in which the rebuilding analysis indicates recovery will occur and the year at or before which it actually occurs; and 3) the probability of being below the overfished level after 20 and 60 years.

The first variant of the baseline management procedure ("with constraints" in Table 4) involves imposing maximum and minimum catch limits of 30 and 8000 t and constraining changes in harvest guideline not to exceed 25% from one year to the next, except in the first year when reductions of up to 99% are allowed. This variant leads to much lower interannual variation in catches when a 60-year period is considered (17% compared with 25%) but the AAV is actually higher for the first 20 years. This variant also leads to higher probabilities of recovery. However, there is still a large discrepancy between the actual year of recovery to $0.4B_0$ and the year that underlies the management procedure (the value of F_{rec} in Table 4 is only 0.27 for the "with constraints" variant).

The second variant considered ("no 10 year and estimated F_{MSY}" see Table 4) drops the requirement that T_{max} be defined as 10 years if the resource can be recovered in 10 years and instead always sets T_{max} to T_{min} plus one mean generation. It also allows the F_{MSY} proxy used when applying the 40-10 rule to differ from the default value of $F_{50\%}$ by setting it to F_{rep} (Jakobsen, 1993) if F_{rep} is lower than F_{MSY}. Estimating (rather than fixing) F_{MSY} is consistent with the recommendation of Brodziak (2002). The major performance difference between this variant and the baseline management procedure is the increased value of F_{rec}.

The "preferred" variant in Table 4 combines the features of the "with constraints" and "no 10 years and estimated F_{MSY}" variants. Compared with the baseline management procedure, it leads to a markedly increased value for F_{rec} (remarkably close, in fact, to the desired value of 0.6), slightly lower catch variability, a less than 5% chance of being overfished after 20 years, and higher probabilities of being recovered to $0.4B_0$ after 20 and 60 years of management. The major disadvantage of this variant is the lower catches and that it leaves the spawning output well above 40% of B_0 after 60 years (see row "preferred" in Table 4).

Prior to the adoption of Amendment 11 of its Groundfish Management Plan, the PFMC set harvest guidelines using only the 40-10 rule.[7] Table 4 therefore also lists results for management procedures based on the 40-10 rule. When the 40-10 rule is applied without any constraints ("PFMC (baseline)" in Table 4), the probability of recovery and the values for the "50%D" statistic are lower (particularly the former) than for the "preferred" variant. In contrast, application of the 40-10 rule with constraints ("PFMC (preferred)" in Table 4) leads, arguably, to no more than a slight difference in catch (the 40-10 rule achieves higher catches) and probability of recovery (the "preferred" variant achieves a higher probability of recovery). The remaining analyses of this paper focus on the "preferred" variant. Future consideration of management procedures for West Coast groundfish resources should consider a management procedure that is based simply on the 40-10 rule and has no associated rebuilding analysis component, at least for comparative purposes. At present, however, such a management procedure would be inconsistent with the SFA because it would not specify the time to recover to the proxy for B_{MSY} (even if the results of this paper suggest that there is considerable uncertainty associated with the estimation of this particular quantity).

Sensitivity to alternative operating model specifications

The values assumed for h and σ_R in the baseline operating model are somewhat arbitrary. Table 5 therefore examines the sensitivity of the results for the "preferred" management procedure to varying the values assumed for these parameters, as well as that of the size of spawning output at the start of the first projection year to B_0.

The results are, as expected, sensitive to all three of the factors considered. Increasing σ_R from 0.4 through 0.6 to 1 leads to lower and more variable catches, a slightly higher probability of recovery in the first 20 years and a markedly higher value of 50%D after 60 years (0.74 for σ_R=1 compared to 0.46 for σ_R=0.4). The ability to detect an overfished stock declines slightly as the extent of variation in recruitment increases. The management procedure behaves as expected as steepness is increased from 0.25 through 0.4 to 0.7; the probability of recovery is markedly higher for high values of steepness even though the management procedure does identify cases with low steepness, and accordingly sets very low harvest guidelines in such cases. However, it is perhaps noteworthy that the probability of correctly identifying that the resource is overfished is lowest for the least productive scenario. The catches for the scenario in which the spawning output is 10% of B_0 at the start of the first projection year are much lower than for the baseline scenario, particularly over the first 20 years. However, these lower catches are necessary to achieve recovery (the median value of the statistic 50%D after 60 years is 0.52 and there is a 0.93 probability of the spawning output having recovered to $0.4B_0$ after 60 years for this scenario).

The behavior of the management procedure can be evaluated in terms of whether it eventually allows the stock to recover to $0.4B_0$ and whether it keeps the stock away from the overfished level of $0.25B_0$. The "preferred" management procedure can be argued to satisfy this criterion, except possibly for the scenario with the lowest steepness but, even in this case, the probability of recovery is 0.6 after 60 years.

The value for the F_{rec} statistic varies markedly depending on steepness and the ratio of the spawning output at the start of the first projection year to B_0. Although the "preferred" management procedure performs well for the baseline scenario in terms of recovering the resource by the predicted value for T_{max}, this good performance is clearly a fortunate anomaly. However, it does help to highlight that predictions of the year-to-recovery from rebuilding analyses should be interpreted with considerable caution.

Sensitivity to data quality

The data-related specifications for the baseline trial (Table 2) could be considered to be data-rich. It is therefore

---

[7] Albeit with different target fishing mortality levels.

Table 5

Performance statistics (see Table 3 for definitions) for 10 variants of the baseline operating model. All of the calculations in this table relate to the preferred management procedure. N/A = not applicable.

				Results after 20 years					Results after 60 years				
Operating model scenario	F_{rec}	Y_{rec}	P_{decl}	5%D	50%D	AAV	$\bar{C}$	P_{rec}	5%D	50%D	AAV	$\bar{C}$	P_{rec}
Baseline	0.59	62	0.82	0.25	0.39	0.31	950	0.49	0.28	0.54	0.21	1961	0.96
Structural changes													
σ_R=0.4	0.59	63	0.86	0.24	0.38	0.26	1242	0.44	0.25	0.46	0.18	2379	0.87
σ_R=1	0.59	61	0.72	0.23	0.41	0.43	417	0.54	0.32	0.74	0.32	592	0.96
h=0.25	0.15	94	0.76	0.20	0.28	0.76	86	0.02	0.23	0.38	0.50	126	0.60
h=0.7	0.84	53	0.87	0.31	0.46	0.16	3427	0.93	0.40	0.61	0.14	3951	1.00
Initial spawning out = 0.1 B_0	0.42	72	1.00	0.19	0.29	0.43	417	0.05	0.27	0.52	0.23	1375	0.93
Initial spawning out = 0.4 B_0	N/A	N/A	N/A	0.31	0.50	0.21	2881	0.92	0.30	0.66	0.19	2849	0.97
Data-related changes													
Deterministic data	0.68	61	0.84	0.29	0.38	0.30	957	0.51	0.31	0.55	0.20	2050	0.98
n^e=50	0.68	60	0.82	0.26	0.39	0.32	785	0.56	0.29	0.55	0.22	1938	0.97
σ^c=1	0.56	62	0.79	0.20	0.39	0.31	987	0.48	0.31	0.57	0.22	1962	0.97
5-yr update frequency	0.55	62	0.80	0.21	0.38	0.27	1160	0.49	0.27	0.53	0.19	1980	0.95

important to assess the sensitivity of the results to the quality of the data. The row "deterministic data" in Table 5 provides results for a trial in which the survey biomass index, the catch-rate index, and the age-composition data are known without error. The results from this trial provide an upper bound on the impact of improved data quality on the assessment results.[8] Somewhat surprisingly, the results for this trial are not notably better than for the baseline trial—the most notable difference between the baseline trial and the "deterministic data" trial being the higher values for the "5%*D*" statistics for the latter trial. The lack of major improvement in performance arises because, even with perfect information on spawning output and recruitment, it is still not possible to estimate B_0 exactly by multiplying average recruitment for the first 10 years of the assessment period by spawning output-per-recruit in the absence of fishing (hence the value of 0.84 for P_{decl}). Furthermore, the rebuilding analyses are still based on generating future recruitment by using spawning output and recruitment data for only 20 years, which is clearly a major source of variability in the predictions from the rebuilding analysis.

Decreasing the catch-at-age sample size from 200 to 50 has relatively little impact on the values for the performance statistics (the AAV statistic is marginally higher and the average catch, particularly for the 20-year projection horizon, is lower). Decreasing the precision of the catch-rate data has a rather larger impact. This is most evident in the value for the "5%D" statistic which is 0.2 rather than 0.25, as is the case for the baseline trial. The "5-yr update frequency" scenario in Table 5 examines the implications conducting assessments every fifth rather than every third year. The results are not markedly sensitive to the interassessment period although the lower values for the "5%D" statistics are perhaps noteworthy.

General remarks

The framework developed in this paper provides an objective basis for contrasting different management procedures and evaluating their sensitivity to uncertainty. Given such a framework, it becomes possible to compare variants of one class of management procedure (e.g. Table 4) and to compare variants among different classes of management procedure.

The management procedure options presented in this paper are but a small subset of those possible. In particular, it should be possible to improve performance by modifying the approach used to generate future recruitment when conducting rebuilding analyses to make use of some form of stock-recruitment relationship. One reason for expected improved performance is that it may then be feasible to estimate the fishing mortality rate corresponding to $0.4B_0$ rather than having to set it to the default value of $F_{50\%}$ or basing it on F_{rep}. Other possible management procedure options include 1) not increasing the rebuilding fishing mortality rate selected when the rebuilding analysis was first conducted if a stock is recovering faster than initially anticipated; 2) not decreasing the rebuilding fishing mortality rate as long of the probability of recovery by T_{max} is at least 0.5; and 3) smoothing the discontinuity that arises when a stock changes status from being under a rebuilding plan to being managed with the 40-10 rule when the stock has recovered to $0.4B_0$. In terms of the last option, one of the issues considered an early rebuilding analysis for widow rockfish involved fishing mortality increasing to its target level as the stock approaches $0.4B_0$ (MacCall[9]).

---

[8] The assessment still ignores interannual changes in selectivity; therefore the assessment results will not be exactly the same as the true values.

The values for the F_{rec} statistic highlight that the predictions of the time to recovery (even in a probabilistic sense) from rebuilding analyses are highly uncertain. The uncertainty of this estimate of the time to recovery is due to the uncertainty about current stock size and that associated with making long-term predictions based on a short time-series of spawning output and recruitment data.

Although the performance of the management procedures is less than ideal, the results are almost certainly optimistic because the operating model is extremely simple and considers no major structural uncertainties (except for variability in selectivity over time). In contrast, Punt et al. (2002) found that including spatial structure in an operating model and assessing the stock by using a spatially aggregated assessment approach led to assessments that were markedly in error. However, the simulations conducted by Punt et al. (2002) were developed for a far more data-poor situation than that for West Coast groundfish, although there is also clearly spatial structure in the West Coast groundfish fishery. Another source of uncertainty not considered in this paper but that may be of critical importance to the management of West Coast groundfish species is the impact of environmental regime shifts, which have been argued to impact long-term trends in recruitment (e.g. Francis et al., 1998).

An important aspect of this study is the ability to focus on the relationship between the overall performance of a management procedure and the performance of its constituent parts. For example, the results for the "deterministic data" scenario in Table 4 show that given the approach used to conduct the future projections, even perfect information from surveys and very large age-composition samples are unlikely to lead to marked improvements over the current situation if that situation is adequately modeled by the baseline operating model. Identification of the key sources of uncertainty could be used to focus future management-related research activities.

The computational requirements of the calculations outlined above are substantial. In particular, the need to apply a fairly complicated method of stock assessment once every three years means that rapid evaluation of management procedures is (currently) computationally not feasible. It is possible, in principle, to simplify the management procedure considerably by assuming that the results from a stock assessment can be mimicked by generating a biomass estimate based on the "true" biomass but with some random error (e.g. Hilborn et al., 2002). However, although such an approach may be satisfactory for some management procedures (e.g. those that set the harvest guideline equal to some fraction of the current biomass), this is not the case for PFMC-type management procedures that depend on the (assessed) age-structure of the population.

It needs to be recognized that any simulation study is by design case-specific. However, the conclusions of this study may be relevant to a fairly broad set of West Coast rockfish species owing to their similar biology and exploitation history—the two factors most likely to impact the relative performance of different management procedures.

Acknowledgments

Discussions with Alec MacCall, John DeVore, and Richard Methot are gratefully acknowledged as are the comments on an earlier version of this paper by Pamela Mace and two anonymous reviewers. This work was funded through NMFS grant NA07FE0473.

Literature cited

Barnes, W. R.
1999. Viewpoint: an industry view of the application of operational management procedures to setting total allowable catches for the South African pelagic fishery. ICES. J. Mar. Sci. 56:1067–1069.

Beddington, J. R., and J. G. Cooke.
1983. The potential yield of fish stocks. FAO Fish. Tech. Pap. 242:1–47.

Brodiak, J.
2002. In search of optimal harvest rates of west coast groundfish. N. Am. Fish. Manage. 22:258–271

Butterworth, D. S., and M. O. Bergh.
1993. The development of a management procedure for the South African anchovy resource. *In* Risk evaluation and biological reference points for fisheries management (S. J. Smith, J. J. Hunt, and D. Rivard, eds.), p. 83–89. Can. Spec. Publ. J. Fish. Aquat. Sci. 120.

Cochrane, K. L., D. S. Butterworth, J. A. A. De Oliveira, and B. A. Roel.
1998. Management procedures in a fishery based on highly variable stocks and with conflicting objectives: experiences in the South African pelagic fishery. Rev. Fish. Biol. Fish. 8:177–214.

Dorn, M. W.
2002. Advice on west coast rockfish harvest rates from Bayesian meta-analysis of stock-recruit relationships. N. Am. Fish. Manage. 22:280–300.

Francis, R. I. C. C.
1992. Use of risk analysis to assess fishery management strategies: a case study using orange roughy (*Hoplostethus atlanticus*) on the Chatham Rise, New Zealand. Can. J. Fish. Aquat. Sci. 49:922–930.

Francis, R. S., A. Hare, A. Hollowed, and W. Wooster.
1998. Effects of interdecadal variability on the oceanic ecosystems of the NE Pacific. Fish. Oceanogr. 7:1–21.

Geromont, H. F., J. A. A. De Oliveira, S. J. Johnston, and C. L. Cunningham.
1999. Development and application of management procedures for fisheries in southern Africa. ICES J. Mar. Sci. 56:953–966.

Hilborn, R.
1979. Comparison of fisheries control systems that utilize catch and effort data. J. Fish. Res. Board Can. 36: 1477–1489.

---

[9] MacCall, A. D. 2002. Personal commun. NMFS Santa Cruz Laboratory, 110 Shaffer Rd, Santa Cruz, CA 95060.

Hilborn, R., A. Parma, and M. Maunder.
2002. Exploitation rate reference points for west coast rockfish: are they robust and are there better alternatives? N. Am. Fish. Manage. 22:365–375.

Ianelli, J. N.
2002. Simulation analyses testing the robustness of productivity determinations from West-Coast Pacific ocean perch stock assessment data. N. Am. Fish. Manage. 22: 301–310.

IWC (International Whaling Commission).
1992. Report of the Fourth Comprehensive Assessment Workshop on Management Procedures. Rep. Int. Whal. Comm. 42:305–321.
2001. Report of the standing working group on the development of an Aboriginal subsistence whaling management procedure. J. Cetacean Res. Manag. 4(suppl.):148–177.

Jakobsen, T.
1993. The behaviour of F_{low}, F_{med} and F_{high} in response to variation in parameters used for their estimation. *In* Risk evaluation and biological reference points for fisheries management (S. J. Smith, J. J. Hunt, and D. Rivard, eds.), p. 119–125. Can. Spec. Publ. J. Fish. Aquat. Sci. 120.

Methot, R. D.
2000. Technical description of the stock synthesis assessment program, 56 p. NOAA Tech. Memo., NMFS-NWFSC-43.

Patterson, K. R., and G. P. Kirkwood.
1995. Comparative performance of ADAPT and Laurec-Shepherd methods for estimating fish population parameters and in stock management. ICES J. Mar. Sci. 52: 183–196.

Punt, A. E., A. D. M. Smith, and G. Cui.
2001. Review of progress in the introduction of management strategy evaluation (MSE) approaches in Australia's South East Fishery. Mar. Freshw. Res. 52:719–726.
2002. Evaluation of management tools for Australia's South East Fishery. 2. How well do commonly-used stock assessment methods perform? Mar. Freshw. Res. 53:631–644.

Restrepo, V. R., and J. E. Powers.
1999. Precautionary control rules in US fisheries management: specification and performance. ICES. J. Mar. Sci. 56:846–852.

Rosenberg, A. A., and S. Brault.
1993. Choosing a management strategy for stock rebuilding when control is uncertain. *In* Risk evaluation and biological reference points for fisheries management (S. J. Smith, J. J. Hunt, and D. Rivard, eds.), 243–249. Can. Spec. Publ. J. Fish. Aquat. Sci. 120.

Sampson, D. B., and Y. Yin.
1998. A Monte Carlo evaluation of the stock synthesis assessment program. *In* Fishery stock assessment models (F. Funk, T. J. Quinn II, J. Heifetz, J. N. Ianelli, J. E. Powers, J. F. Schweigert, P. J. Sullivan, and C. I. Zhang, eds.), p. 315–338. Alaska Sea Grant, Fairbanks, AK.

Starr, P. J., P. A. Breen, R. H. Hilborn, and T. H. Kendrick.
1997. Evaluation of a management decision rule for a New Zealand rock lobster substock. Mar. Freshw. Res. 48: 1093–1101.

Appendix 1 : An overview of the technical aspects of the PFMC's rebuilding analysis

The key steps of the PFMC's rebuilding analysis are 1) to select the maximum allowable rebuilding time (T_{max}), 2) to develop specifications for projecting the population size at the start of the current year-to-year T_{max}, and 3) to calculate the target fishing mortality rate so that the probability of the spawning output rebuilding to $0.4B_0$ at or before T_{max} equals a prespecified value, p_{rec} (taken to be 0.6 for purposes of the present study).

Projecting the population forward and defining B_0

The population projections are based on the equation

$$N_{y,a} = \begin{cases} R_y & \text{if } a = a_{\min} \\ N_{y-1,a-1}e^{-(M+S_{a-1}F)} & \text{if } a_{\min} < a < a_{\max} \\ N_{y-1,a_{\max}-1}e^{-(M+S_{a_{\max}-1}F)} + N_{y-1,a_{\max}}e^{-(M+S_{a_{\max}}F)} & \text{if } a = a_{\max}, \end{cases} \quad (\text{A.1})$$

where $N_{y,a}$ = the number of animals of age a at the start of year y;
M = the instantaneous rate of natural mortality (assumed to be independent of age);
S_a = the selectivity for animals of age a;
F = the fully selected (i.e. $S_a \rightarrow 1$) fishing mortality;
R_y = the recruitment (both sexes) during year y;
a_{min} = the lowest age class considered in the model; and
a_{max} = the oldest age class considered in the model (treated as a plus-group).

The age structure of the population at the start of the first year of the projection period is taken to be that from the most recent assessment. A variety of approaches are available to generate future recruitment (PFMC[10]). However, for consistency with the approach used in the bulk of the rebuilding analyses conducted to date, future recruitment is either based on randomly sampling recruitments (with replacement) from a prespecified historical period or based on randomly sampling the ratio of the recruitment to the spawning output that spawned that recruitment (with replacement) and then multiplying by current spawning output. The choice between basing the projections on sampling recruitments or sampling recruits-per-spawning output is determined by regressing each of these on time and selecting whichever has the lesser slope. The reason for doing this is that the lack of a trend in recruits-per-spawning output is indicative of a stock-recruitment relationship with low "steepness" (Francis, 1992), whereas the lack of a trend in recruitment is indicative of a stock-recruitment relationship with high "steepness."

The pre-exploitation equilibrium spawning output used to determine the rebuilding target is computed by multiplying the unfished spawning output-per-recruit by the average recruitment over a prespecified number of historical years. Note that the range of years on which to base the estimate of B_0 will usually differ from that on which generation of future recruitment is based.

---

[10] PFMC (Pacific Fishery Management Council). 2001. SSC terms of reference for groundfish rebuilding analysis, 9 p. Pacific Fishery Management Council, 7700 NE Ambassador Place, Portland, OR 97220.

It should also be noted that no account is taken of uncertainty regarding the current age structure, natural mortality, selectivity, etc., although the projections do account for uncertainty about future recruitment

Selecting the maximum allowable rebuilding period

The maximum allowable rebuilding time, T_{max}, is defined as the maximum of 10 years and the sum of the mean generation time and the minimum possible rebuilding time. This specification implements the requirement of the SFA to "take into account the status and biology of any overfished stocks of fish, [and] the needs of fishing communities." The minimum possible rebuilding period for a given future projection is computed by projecting the population forward with zero fishing mortality and by identifying the year in which the spawning output first reaches $0.4B_0$. T_{min} is the median of the distribution for this year constructed by conducting projections for many different (random) realizations of future recruitment.

Calculating the target fishing mortality rate

The target fishing mortality rate and hence the harvest guideline are determined by projecting the population forwards many times (100 times for the purposes of this paper), each time with a different sequence of future recruitment and for a variety of alternative Fs and then identifying the level of F that corresponds to the spawning output having reached $0.4B_0$ by T_{max} with the prespecified probability p_{rec}.

Distribution, demography, and discard mortality of crabs caught as bycatch in an experimental pot fishery for toothfish (*Dissostichus eleginoides*) in the South Atlantic

Martin G. Purves
Marine and Coastal Management
P.O. Box X2, Roggebaai 8012
Cape Town, South Africa
Present address: Resources Assessment Group
47 Prince's Gate
London SW7 7QA, United Kingdom
E-mail address: m.purves@imperial.ac.uk

David J. Agnew
Renewable Resources Assessment Group
Imperial College
Royal School of Mines
Prince Consort Road
London, SW7 2BP, United Kingdom

Guillermo Moreno
Tim Daw
MRAG Ltd
47 Princes Gate
London, SW7 2QA, United Kingdom

Cynthia Yau
Zoology Department
University of Aberdeen
Aberdeen, AB24 2TZ, United Kingdom

Graham Pilling
Centre for Environment, Fisheries and Aquaculture Science (CEFAS)
Pakefield Road
Lowestoft, NR33 OHT, United Kingdom

Abstract—Between March 2000 and April 2001 two commercial fishing vessels fished for toothfish (*Dissostichus eleginoides*) off South Georgia using pots. A significant number of lithodid crabs (three species of *Paralomis* spp.) were caught as bycatch. *Paralomis spinosissima* occurred in shallow water, generally shallower than 700 m. *Paralomis anamerae*, not previously reported from this area and therefore representing a considerable southerly extension in the reported geographic range of this species, had an intermediate depth distribution from 400 to 800 m. *Paralomis formosa* was present in shallow waters but reached much higher catch levels (and, presumably, densities) between 800 and 1400 m. Differences were also noted in depth distribution of the sexes and size of crabs. Depth, soak time, and area were found to significantly influence crab catch rates. Few crabs (3% of *P. spinosissima* and 7% of *P. formosa*) were males above the legal size limit and could therefore be retained. All other crabs were discarded. Most crabs (>99% of *P. formosa*, >97% of *P. spinosissima*, and >90% of *P. anamerae*) were lively on arrival on deck and at subsequent discard. Mortality rates estimated from re-immersion experiments indicated that on the vessel where pots were emptied directly onto the factory conveyor belt 78–89% of crabs would survive discarding, whereas on the vessel where crabs were emptied down a vertical chute prior to being sorted, survivorship was 38–58%. Of the three, *P. anamerae* was the most vulnerable to handling onboard and subsequent discarding. *Paralomis spinosissima* seemed more vulnerable than *P. formosa*.

Manuscript approved for publication
17 April 2003 by Scientific Editor.
Manuscript received 26 June 2003
at NMFS Scientific Publications Office.
Fish Bull. 101:874–888 (2003).

The Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) and its Scientific Committee were pioneers in the development of the "ecosystem approach" for the management of fisheries. Using this approach the Commission is bound to consider the impact of any fishery on both the target, dependent, and related species. Currently, the most important fishery in CCAMLR waters is the longline fishery for Patagonian toothfish (*Dissostichus eleginoides*) and fishing grounds near South Georgia Island and Shag Rocks in CCAMLR subarea 48.3 (South Atlantic sector) are among the most important. Mitigation measures, including requirements for setting at night, in the winter, and with specialized gear, have been introduced to reduce incidental mortality of birds being hooked by longlines. However, these measures impose severe operational restrictions on the fishery, and low levels of bird mortality still occur (CCAMLR[1]).

Pot fishing for toothfish has recently been tried around South Georgia (Agnew et al., 2001), and although pots do not catch birds, they do take lithodid crabs as bycatch. Crabs are largely a "nuisance" catch when fishing for toothfish, but this bycatch is clearly of concern in relation to crab populations, and must be considered within the CCAMLR's ecosystem approach. A small amount of exploratory crab fishing has already taken place around South Georgia Island and Shag Rocks. Only 798 metric tons (t) of crabs have been taken in directed crab fisheries since 1992; by the FV *Pro Surveyor* (July–August 1992; 299 t; CCAMLR[2]), the FV *American Champion* (September 1995–January 1996; 497 t; CCAMLR[3]), and the FV *Argos Helena* (August 1999; 2 t) (CCAMLR[4]). A pot fishery for toothfish is likely to take place in deep water where current longline fishing concentrates (around the 1000 m contour: Agnew et al., 1999) rather than in shallower waters (<400 m) where crab fishing has taken place (Otto and Macintosh, 1996; Watters, 1997). Toothfish pot fishing may therefore impact different crab population components from those impacted by the crab fishery.

We investigated the likely effects of toothfish pot fishing on crabs caught as bycatch on board two commercial fishing vessels. These vessels conducted three separate trials fishing for toothfish around South Georgia between March 2000 and April 2001. This paper reports on the species of crab taken during these trials, as well as the distribution of crabs and their biological characteristics. In common with many other crab fisheries (Hoggarth, 1991; Schmidt and Pengilly, 1993) retention size limits are set for the fishery around South Georgia. Only males greater than 102 mm carapace width for *Paralomis spinosissima* and 90 mm for *P. formosa* may be retained. All undersize and female crabs from both the toothfish pot fishery and the crab fishery must be discarded. We also report results of experiments on survival of such discards.

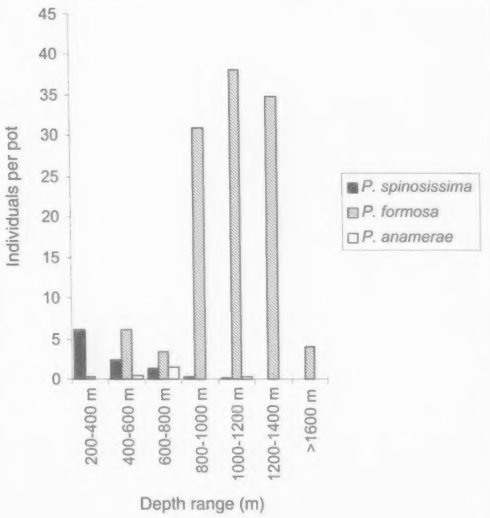


Figure 1

Catch in numbers per pot of the three dominant crab species by depth range as found during fishing operations of the *Argos Georgia* from May to April 2000. Pots were set between 211 m and 1651 m depth.

Methods

During the first cruise (March to May 2000), two observers were deployed on board the FV *Argos Georgia* (cruise G1). Detailed information was collected on the number of toothfish and the numbers and species composition of crabs caught in the pots. Information was also collected on fishing results, such as catch rates, fish bycatch, and the commercial viability of this fishing method (Agnew et al., 2001), and the diet of toothfish (Pilling et al., 2001). In the second and third toothfish pot cruises single observers were deployed on the *Argos Georgia* (cruise G2) and another vessel, the FV *Argos Helena* (cruise H), which fished simultaneously from January to April 2001. Fishing gear and the configuration of gear was similar for all three cruises. The semiconical pots of approximately 80 cm height were constructed of steel frames and covered with 80-mm polysteel (Movline) mesh. A collapsible funnel entrance was situated on the side of the pot, orientated horizontally, and tapering to the pot's interior. A drawstring held the bottom mesh together in the middle. This configuration allowed the pots to be emptied easily when hauled aboard and to be stacked on top of each other when not in use. A panel was sewn into the pots by using biodegradable sisal twine to ensure that crabs could eventually escape from lost pots and to prevent "ghost fishing." However, catch handling methods were different on the two vessels; the pots were emptied directly onto the factory conveyor belt on the *Argos Georgia* and emptied down a chute to the factory level on the *Argos Helena*.

During the first cruise (March to May 2000) depth of fishing, determined as water depth by onboard echo sounders, was related to the species of crab caught. *Paralomis spinosissima* were generally caught in relatively shallow waters, whereas *P. formosa* tended to be caught in much greater numbers in deeper waters (Fig. 1). *Paralomis anamerae*

---

[1] CCAMLR (Commission for the Conservation of Antarctic Marine Living Resources). 1999. Report of the Working Group for Fish Stock Assessment, 110 p. Annex 5 in the report of the eighteenth meeting of the Scientific Committee. CCAMLR, P.O. Box 213, North Hobart, Tasmania 7002, Australia.

[2] CCAMLR. 1992. Report of the working group for fish stock assessment, 164 p. Annex 5 in the Report of the eleventh meeting of the Scientific Committee. CCAMLR, P.O. Box 213, North Hobart, Tasmania 7002, Australia.

[3] CCAMLR. 1997. Report of the working group for fish stock assessment, 169 p. Annex 5 in the Report of the sixteenth meeting of the Scientific Committee. CCAMLR, P.O. Box 213, North Hobart, Tasmania 7002, Australia.

[4] CCAMLR. 2000. CCAMLR statistical bulletin, 153 p. CCAMLR, P.O. Box 213, North Hobart, Tasmania 7002, Australia.

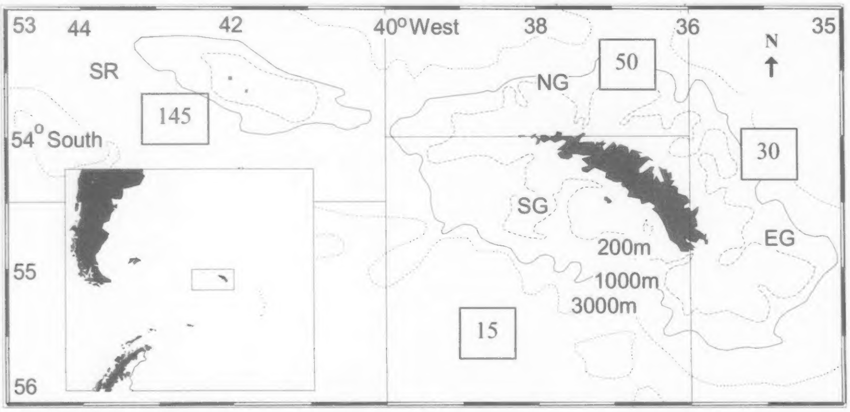


Figure 2

Map of areas used in the study. SR=Shag Rocks, NG=North South Georgia, EG=East South Georgia, SG=South South Georgia, and the 200-m, 1000-m and 3000-m bathymetric contours are shown. The total number of hauls in each of these areas were as follow: SR=145, NG=50, EG=30, and SG=15. Inset shows the position of the study area in relation to the South Atlantic.

(Macpherson, 1988) were caught at intermediate depths. Other influences on crab catch rates were investigated by using two cruises in 2001, which covered the study area more evenly (most hauls in cruise G1 were concentrated in a small area around southeast Shag Rocks; see Fig. 2). For each species two generalized linear models were constructed with Splus statistical software (version 2000, Mathsoft Engineering & Education, Inc., Cambridge, UK): a binomial link model for the probability of encountering a crab of that species (*pe*) and a Gaussian link model for the natural logarithm of CPUE (catch in numbers per pot) for all nonzero catches. Both models were of the form $\lambda = p_1 \times$ depth$+ p_2 \times$area$+ p_3 \times$vessel$+ p_4 \times$soak time, where for the binomial models, λ was set to 1 if crabs had been caught and 0 if they had not, and for the Gaussian models, λ was set to ln(numbers per pot) for all sets catching crabs. Area and vessel parameters were factors. Depth and soak time were modeled as linear continuous variables, except in the case of the Gaussian model for *P. formosa*, where a third-order polynomial best described the relationship between CPUE and depth. Predictions from the two models were combined to predict crab catch rates per pot,

$$\hat{U} = p(e) \times \exp[\hat{u} \pm 1.96 \times SE],$$

where $\hat{u}$ = the predicted ln(CPUE) from the Gaussian model; and

SE = the standard error from the Gaussian model.

Biological data were collected from all crabs in randomly selected pots. Carapace widths, carapace lengths, chela height, and chela length were measured to the nearest millimeter below by using calipers. Weights were measured to the nearest 5 g below with spring-balanced scales. Sex, maturity stage, condition of the carapace, and an index of vitality (Table 1) were recorded for a subsample of crabs, selected as a random portion across species from the contents of selected pots. Identification of the uncommon species, *Paralomis anamerae*, *Neolithodes diomedeae,* and *Lithodes murrayi*, was confirmed by using dried specimens in London (at Imperial College and the British Museum of Natural History).

Male size at maturity was determined from the allometric relationship between carapace length (L) and right (dominant) chela size (height, CH, or length, CL). The slope of the L-CH or L-CL relationship is assumed to change when crabs reach maturity. Around South Georgia, Otto and Macintosh (1996) used L and CH to determine the size at maturity of male *P. spinosissima,* and around the Falkland Islands Hoggarth (1991) used L and CL. For both *P. spinosissima* and *P. formosa* we found that the intersection of the two lines corresponding to the onset of maturity was not easy to identify from the relationship between CL and L. We therefore used CH in our relationships. Following the methods of Somerton and Otto (1986), two linear regression lines were fitted to natural logarithms of L and CH or CL. These lines represent juvenile and adult phases in the L-CH or CL relationship and intersected at a point taken as the L at which males became mature. The regression lines of best fit were determined by minimizing the combined residual sums of squares, and standard errors were estimated by using 500 bootstraps (sampling with replacement).

Females were classified into two categories, "eggs absent" and "eggs present." The vast majority of "eggs absent" females were small immature animals, but some large animals were also encountered in this category. Consequently, for estimating size at maturity, females in the "eggs present" category were defined as "mature" and those in the "eggs absent" category were classified as "immature" up to the size at which the proportion of females with eggs (i.e. mature) reached 90%, after which they were classified as "mature without eggs." Female size at maturity was deter-

Table 1

Relative index used for assessing vitality in *Paralomis* spp.

Vitality index	Description	Characteristics
1	Lively	Limbs supported and held out. Limbs resist manipulation. Crabs actively pinch objects. Can hang on smooth end of forceps by 1 claw (weakest on large crabs).
2	Lively but limp	Legs hang when picked up. Claws weak and can be opened—not capable of supporting own weight on forceps. Mouthparts move, indicating life when submerged in seawater.
3	Dead	No signs of life. Mouthparts do not move when submerged.
4	Dead and eaten	Only shell or carapace remaining.

mined by plotting the proportion of mature females against size (carapace width) and determining the point of 50% maturity (Sm_{50}). Logistic curves of the form [$proportion\ mature = 1/(1 + \exp(-r(carapace\ width - Sm_{50})))$] were used to estimate 50% maturity (Sm_{50}) and its standard error by using the nonlinear fitting function in Splus.

Three different experiments were conducted between March 2000 and April 2001 to assess crab discard survival rates. During cruise G1 a number of alive and active crabs from one haul were tagged through one of the lateral plates of the abdomen with Hallprint plastic T-bar tags and maintained in running seawater before they were placed in pots prior to the next setting. Once these pots were rehauled, the vitality of the tagged crabs was assessed by using the four point relative scale shown in Table 1. A control group of crabs were similarly tagged and kept onboard in running seawater to monitor any effect that tagging might have had on survival. Survival experiments conducted on cruises G2 and H differed in that crabs were selected at random and included individuals that were already "limp" prior to re-immersion. Crabs were tagged with thin strips of masking tape around their legs prior to re-immersion. To ensure that the same crabs were assessed for vitality after rehauling, pots were marked and sealed off to prevent any new captures.

Estimates of the total survival that can be expected after discarding were made in the following manner. By observing crabs on arrival on deck we determined the proportion of animals that arrived on deck as lively ($lively_0$), or limp ($limp_0$), or dead. Using the data from the survival experiments we set *p(lively,lively)* as the probability that a lively animal that is discarded will recover to a lively condition (this was estimated by calculating the proportion of experimentally re-immersed lively animals that were recovered as lively). We defined *p(limp,lively)* similarly. The proportion of discarded animals that were lively and would continue to be lively is $lively_0 \times p(lively,lively)$ and the proportion of discarded animals that were limp but would recover to a lively condition is $limp_0 \times p(limp,lively)$. The overall survivorship, S, is then

$$S = lively_0 \times p(lively,lively) + limp_0 \times p(limp,lively).$$

In our experiments, some of the damage may have occurred on rehauling the pots after re-immersion, a situation that would not normally occur once crabs have been discarded. *p(lively,lively)* can be corrected for this by adding to it the proportion of animals that were not lively when first hauled (i.e. $1-lively_0$), termed the rehaul correction. For instance, suppose that 1% of the crabs were not lively on the first hauling, and in the experiment 4% of the re-immersed lively crabs were not lively on recovery. The rehaul correction would indicate that 1% of the re-immersed crabs would have been damaged anyway simply by the hauling process, and that therefore the correct damage rate attributed simply to the initial capture and discarding would be 3%.

Results

Crab catch

The majority of the bycatch comprised two species of lithodid crabs (Anomura: Lithodidae), *Paralomis spinosissima* and *P. formosa*. Both species have been previously reported in catches around Shag Rocks and South Georgia (Otto and Macintosh, 1996) and have formed a large proportion of the total catch of crab (Table 2). Crab species formed 69.5% of the total weight of all species caught, including toothfish, and 98.2% of the total numbers of individuals caught.

Three other species of crab were also identified during the pot trials. The most abundant of these was *Paralomis anamerae* (Macpherson, 1988), which like the other *Paralomis* species, was subject to detailed biological sampling. 12,370 individuals of this species (1 721 kg) were caught. All individuals were discarded because they were smaller than the minimum size limit for the smaller of the two regulated species, *P. formosa*. Two other species, *Neolithodes diomedeae* and *Lithodes murrayi*, were also caught in small numbers.

Distribution

Crab distribution was investigated for the areas as defined in Figure 2. There were too few data from South of South Georgia; therefore the analysis was restricted to data from Shag Rocks, North South Georgia, and East South Georgia. A few pot strings had been left for several days because

Table 2

Species proportion (no. of crabs and percentage) and discard rates for crabs caught during the pot trials around South Georgia during the period March 2000 to April 2001.

	Number kept	Number discarded	% total crab catch by number	% total crab catch by weight	Discard rate
Paralomis formosa	22,803	300,660	70.1%	63.1%	93%
Paralomis spinosissima	3576	121,580	27.1%	35.4%	97%
Paralomis anamerae	0	12,370	2.7%	1.4%	100%
Neolithodes diomedeae	0		<0.1%	<0.1%	
Lithodes murrayi	0		<0.1%	<0.1%	

Table 3

The results of fitting generalized linear models on the probability of encountering crabs, and the catch rate of crabs (numbers per pot) for nonzero catches. ANOVA results: the significance of adding each parameter to the general linear model is presented. For the binomial model of the probability of encounter, a chi-squared significance test was used; for the Gaussian model of ln(CPUE) an F test was used. Coefficients $p_1..p_4$ are given with standard error in parentheses. For those models including "area" the standard case was for East South Georgia (EG), and the parameters for North South Georgia and Shag Rocks (NG, SR) are given. Analyses were performed with Splus statistical software. Final models were constructed by using only the significant parameters (italicized in this table). n = number of crabs in sample. Poly (1), Poly (2), and Poly (3) are the coefficients of each of the three orders in a 3rd order polynomial.

Parameter	Probability of encountering *P. formosa*	Ln(CPUE) for nonzero catches of *P. formosa*	Probability of encountering *P. spinosissima*	Ln(CPUE) for nonzero catches of *P. spinosissima*
ANOVA results				
n	101	91	101	82
Depth (m)	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Area	>0.05	>0.05	>0.05	*<0.01*
Vessel	>0.05	>0.05	>0.05	>0.05
Soak time (h)	>0.05	*<0.05*	>0.05	*<0.01*
Coefficients $p_1.p_4$				
n	101	91	101	82
Intercept	−2.2358 (1.4373)	1.7229 (0.4301)	8.5065 (1.5822)	3.217 (0.4877)
Depth (m)	0.0098 (0.0035)	Poly(1): 4.6387 (5.3605) Poly(2): −8.4647 (6.2257) Poly(3): −1.3614 (4.4808)	−0.0106 (0.0022)	−0.0055 (0.0008)
Area				NG: 1.1304 (0.3241) SR: 1.2771 (0.2875)
Soak time (h)		0.0447 (0.0221)		0.0638 (0.0191)

of bad weather, and to eliminate these the maximum soak time was limited to 39 hours. The mid-depth of the set (average of the depth at the start and end of the set) was used to indicate setting depth, and the analysis was restricted to sets whose depth range was less than 200 m. The data from 45 sets (19% of total) were omitted from analyses because they did not meet these criteria for area, depth, or soak time.

For the binomial encounter models, the only significant factor was depth (Table 3). For the Gaussian catch per pot model, depth, and soak time were significant for both species and area was significant for *P. spinosissima*. However, the depth effects were opposite for the two species, so that *P. spinosissima* decreased in abundance with depth and *P. formosa* increased in abundance with depth, at least up to about 1000 m depth (Fig. 3). There were insufficient data from the cruises in 2001 to establish the effect of depths shallower than 300 m and deeper than 1100 m, although the limited sampling at depths greater than 1200 m in year 2000 (Fig. 1) suggests that catch rates of *P. formosa* would continue to decline at these depths, as suggested by the generalized linear models in Figure 3.

The sex ratio of *P. formosa* was skewed towards males in shallow water (<800 m) and females in deep water (Fig. 4). The mean size of *P. formosa* of both sexes also decreased significantly with an increase in depth (Fig. 5). Although catch rates in numbers were usually much higher in deeper water, smaller crabs of no commercial value often dominated catches. Only about 38% of *P. spinosissima* sampled between 200 and 400 m were males, whereas this proportion increased to about 76% in depths of 600 to 800 m (Fig. 4). The mean carapace width of males remained relatively constant between 200 to 800 m, but female *P. spinosissima* decreased in size with an increase in depth (Fig. 5).

Size frequencies

Males achieved larger sizes than females in all three species (Fig. 6). Only 5.7% of the sampled *P. spinosissima* individuals were of legal size (carapace width greater than 102 mm), of which only 6% were females. A difference was also noted in the percentage of legal-size *P. spinosissima* for the different areas: 10% at South Georgia and only 3.8% at Shag Rocks. For *P. formosa* only 11.6% (n=1012) were larger than the minimum legal size of 90 mm. Of these legal crabs only 6% (n=63) were females, indicating that few females would be processed if carapace width was the only criterion used to select crabs that could be taken legally. No obvious difference was noted in the percentage of legal-size crabs caught in the two main fishing areas.

Although a legal-size limit is not specified for *P. anamerae* (sizes ranged from 39 to 96 mm), only two crabs (0.2% of sample) were larger than the legal limit for *P. formosa* (90 mm). A peak in the length distribution of this species occurred between 55 and 57 mm, and few crabs were larger than 77 mm.

Differences in the size-frequency distribution of the sexes was more pronounced for *P. formosa* than for the other two species; females peaked at 65–72 mm and males peaked at between 85 and 90 mm carapace width (Fig. 6). For *P. spinosissima* female size distribution peaked between 78 and 82 mm and males peaked at 87 to 92 mm carapace width (Fig. 6). Males and females of *P. anamerae* had a relatively even size distribution up to carapace widths of 65 mm. Most of the larger crabs were males; only 6.8% of individuals larger than 65 mm were females. Maximum widths recorded were 121 mm (*P. spinosissima*), 120 mm (*P. formosa*), and 91 mm (*P. anamerae*).

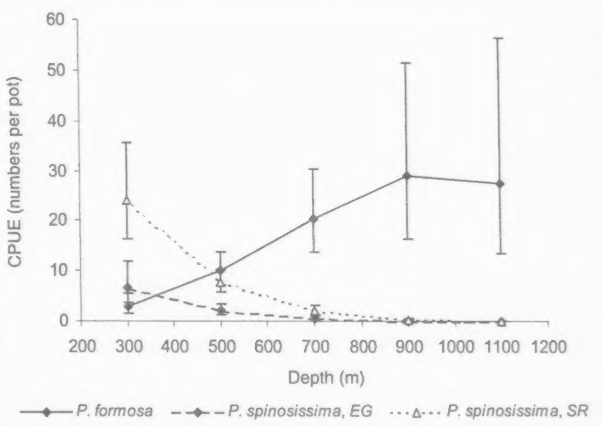


Figure 3

General-linear-model–predicted CPUE (numbers per pot) for the two species (*Paralomis formosa* and *P. anamerae*) at different depths standardized for a soak time of 18 h (the average used in the study). For *P. spinosissima* predictions at East South Georgia and Shag Rocks are presented separately (EG, SR, respectively).

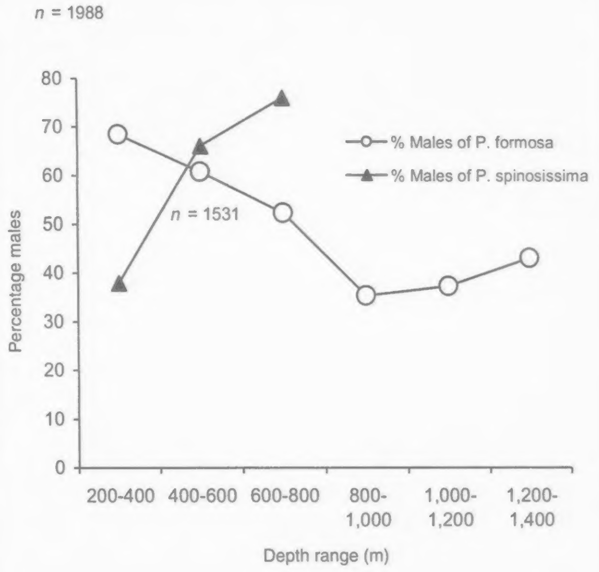


Figure 4

The percentage of males of *Paralomis formosa* and *P. spinosissima* found at different depth ranges during random sampling of the crab bycatch; all data from cruises G1 (*Argos Georgia* cruise 1), G2 (*Argos Georgia* cruise 2), and H (*Argos Helena* cruise) were used.

Size at maturity

There was no significant difference between female size at maturity at Shag Rocks and South Georgia for either species (t-tests on Sm_{50} estimated by fitting logistic models to the proportion mature at size, P>0.05). The close allometric relationship found between carapace width and length (Table 4) made it possible to alter between these two types of measurements (Table 5). Combining the two areas, female size at maturity was 55.1 mm carapace length (57.1 mm carapace width) for *P. formosa* and 61.2 mm carapace length (67.7 mm carapace width) for *P. spinosissima* (Fig. 7). These sizes are very similar to the 61.7 mm

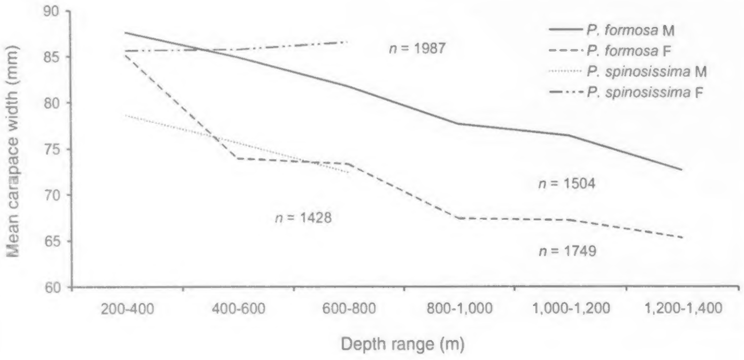


Figure 5

Mean carapace width of the males (M) and females (F) of *P. formosa* and *P. spinosissima* found at different depth distributions.

Table 4

Parameters of the regression *carapace width* = *carapace length* × slope + intercept (all ages and sexes combined). *n* = number of crabs in sample.

Species	Intercept	Slope	Correlation coefficient	*n*
P. spinosissima	6.457	0.994	0.977	367
P. formosa	1.976	1.032	0.969	351
P. anamerae	5.700	0.917	0.979	28

Table 5

The size at maturity (Sm_{50}) for males and females of both *Paralomis* species. The standard errors (SE) for male carapace lengths are shown in parentheses.

	P. spinosissima		*P. formosa*	
	Carapace length (mm)	Carapace width (mm)	Carapace length (mm)	Carapace width (mm)
Males	67.3 (0.10)	73.4	64.0 (0.16)	68.0
Females	61.2	67.7	55.1	57.1

carapace length reported by Otto and MacIntosh (1996) for *P. spinosissima* from Shag Rocks and South Georgia.

Unfortunately, owing to limited sampling of male crabs around South Georgia, estimates of male sexual maturity were only available for Shag Rocks. Too few samples of *P. anamerae* were available for determination of the onset of female or male maturity in either area. Male size at maturity (Sm_{50}) was determined at a carapace length of 67.3 mm (SD=2.3 mm derived from bootstrap resampling with replacement) for *P. spinosissima* and at 64.0 mm (SD= 3.6 mm) for *P. formosa* (Fig. 8).

Crab survival rate

Approximately 5000 crabs were examined for carapace damage on the cruises in 2001. The results (Table 6) indicated that the level of visible damage to these crabs prior to discarding was very low (2% of crabs of all species). The vitality of these crabs was also assessed (according to the index in Table 1). Most crabs were lively on arrival on deck and prior to discard (Table 6). Differences were, however, noted in both carapace condition and the vitality of crabs between the two fishing vessels. Pairwise χ^2 comparisons

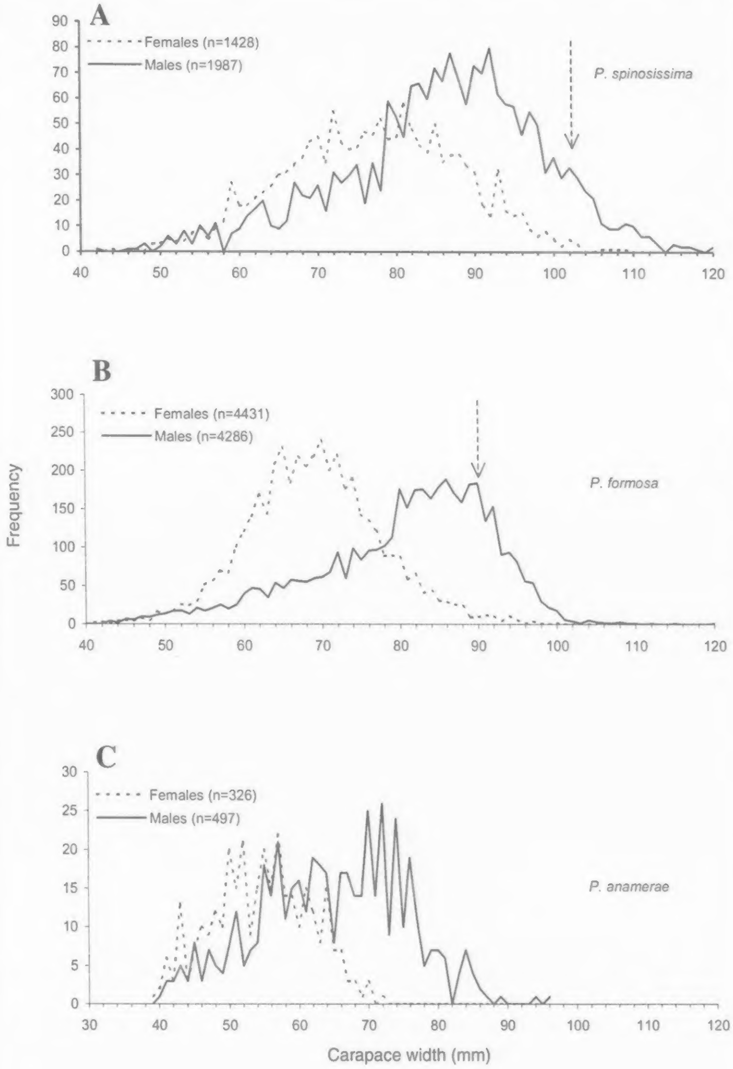


Figure 6

Carapace-width frequency distribution for the three *Paralomis* species (mm). (**A**) *P. spinosissima*, (**B**) *P. formosa*, (**C**) *P. anamerae*. The minimum legal size (102 mm for *P. spinosissima* and 90 mm for *P. formosa*) is indicated with a vertical line and arrow.

between the vitality indices of *P. formosa* and *P. spinosissima* clearly showed that both species displayed significantly lower vitality on the *Argos Helena* than on the *Argos Georgia* (Table 7).

The processing environment of the two vessels may explain these differences. On the *Argos Helena* crabs were likely to sustain more damage as pots were emptied down a vertical chute before entering the processing area below deck. On the *Argos Georgia* pots were emptied on a horizontal conveyer belt leading to the factory. Interestingly, there was no significant difference between the vitality displayed by *P. anamerae* between the two vessels, although this may be a result of the smaller sample size for this species. For the *Argos Georgia*, the vitality of *P. anamerae* was significantly lower than for either *P. formosa* or *P. spinosissima*.

The results of the three survival experiments are shown in Table 8. Experiment 1 re-immersed only lively crabs and included a control set of animals retained on deck in a large tank for the same length of time as the re-im-

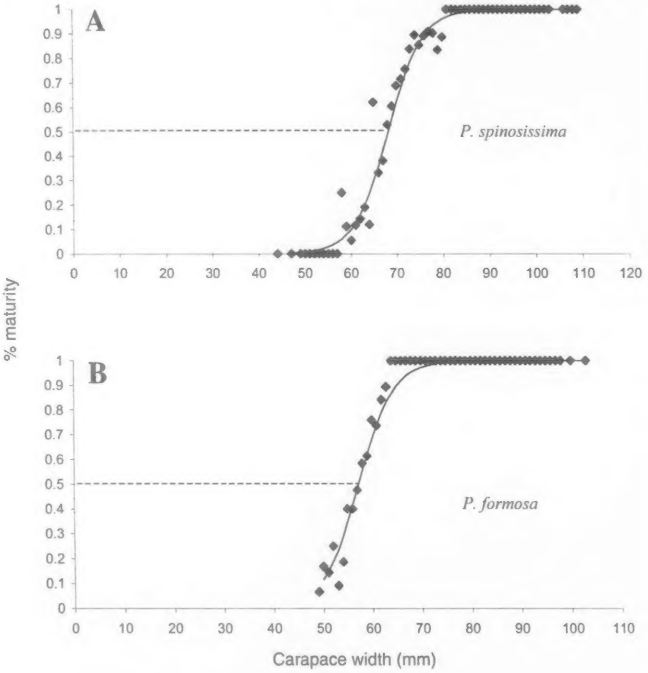


Figure 7

Size at female maturity for (**A**) *P. spinosissima* and (**B**) *P. formosa*. The size at maturity $L(_{50})$ carapace width was 67.7 mm and 57.1 mm for the two species, respectively.

Table 6

The number and percentages of crabs of the different species assessed for carapace condition and vitality prior to being discarded during 2001 (G2=*Argos Georgia* cruise no. 2, H=*Argos Helena*).

	Carapace condition					Vitality index						
	Damaged		Undamaged			Lively		Limp		Dead		
Species and cruise	No.	%	No.	%	Total	No.	%	No.	%	No.	%	Total
Paralomis formosa												
G2	48	1.9	2431	98.1	2479	2294	98.9	26	1.1	0	0.0	2320
H	23	2.7	814	97.3	837	745	97.4	19	2.5	1	0.1	765
Total	71	2%	3245	98%	3316	3039	98%	45	1%	1	0%	3085
Paralomis spinosissima												
G2	17	1.5	1143	98.5	1160	1122	98.4	17	1.5	1	0.1	1140
H	18	2.0	874	98.0	892	767	94.8	39	4.8	3	0.4	809
Total	35	2%	2017	98%	2052	1889	97%	56	3%	4	0.2%	1949
Paralomis anamerae												
G2	0	0.0	45	100.0	45	31	86.1	5	13.9	0	0	36
H	2	2.7	72	97.3	74	62	92.5	5	7.5	0	0	67
Total	2	2%	117	98%	119	93	90%	10	10%	0	0%	103

Table 7

Results of pairwise χ^2 comparisons of the vitality of crabs on first being caught by different cruises (from Table 6). G2 = *Argos Georgia* cruise no. 2 and H = *Argos Helena*, F = *P. formosa*, S = *P. spinosissima*, A = *P. anamerae*. χ^2 values are given, together with significance, df = 2 for all except italicized results, when df was 1 because of the absence of any dead crabs in the comparisons. NS = not significant.

	G2-F	H-F	G2-S	H-S	G2-A
G2-F					
H-F	10.49, $P<0.01$				
G2-S	2.9, NS	—			
H-S	—	6.99, $P<.05$	20.7, $P<0.001$		
G2-A	*35.2, P<0.001*	—	29.2, $P<0.001$	—	
H-A	—	5.5, NS	—	1.14, NS	*0.49, NS*

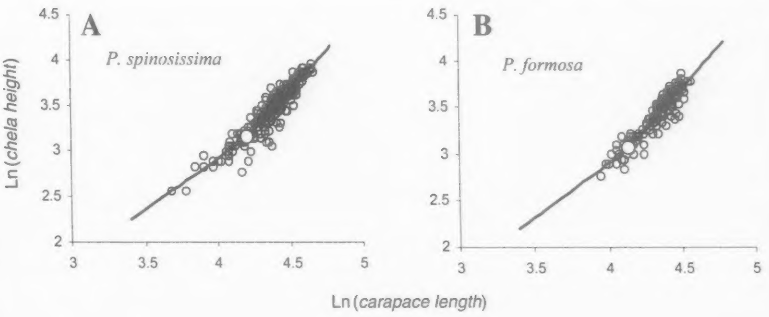


Figure 8

Chela allometry in male (**A**) *P. spinosissima* and (**B**) *P. formosa* sampled at Shag Rocks in 2001. The intersections of fitted curves were used to determine size at maturity.

mersion process. Out of the 35 lively control animals a similar proportion died during the experiment as in the re-immersed group (8%), but a lower proportion of the control group were lively following the experiment (63%). The lower proportion of lively animals in the control group may have been a result of interruptions in the supply of oxygenated water and the continual disturbance on deck due to the ship's motion. Consequently, controls were not performed in experiments 2 and 3.

Experiments 2 and 3 took a random sample of lively and limp crabs and subjected them to re-immersion. The proportions of lively crabs at the beginning of the re-immersion experiments were lower than the proportions estimated for the population as a whole in Table 6, with the exception of *P. spinosissima* in experiment 2. However, sample sizes were much smaller in the experiments and the crabs were subject to greater handling times than those assessed in Table 6; therefore the results presented in Table 6 are more likely to be representative of the condition of discarded crabs than results presented in Table 8.

In experiment 1 on cruise G1, no limp animals were subjected to re-immersion. In comparing the results of immersing lively animals, there was no significant difference between the results for experiment 1 and 2 (cruises G1 and G2 on the *Argos Georgia*) for either *P. formosa* or *P. spinosissima*. Combining the results of the experiments for G1 and G2, there was a significantly lower vitality after re-immersion for both species on the *Argos Helena* compared with the *Argos Georgia* (Table 7). On the *Argos Georgia* there was no significant difference in vitality after re-immersion between *P. formosa* and *P. spinosissima*, whereas on the *Argos Helena* vitality for *P. spinosissima* was significantly lower than vitality for *P. formosa*. These results are similar to the initial assessment of vitality prior to re-immersion (Table 6), where significantly fewer *P. spinosissima* were lively in comparison with *P. formosa* on the *Argos Helena*, but there was no difference between the two species on the *Argos Georgia*.

A single re-immersion experiment performed on 15 *P. anamerae* crabs (11 were "lively" and 4 were "limp"), on the *Argos Helena* in April 2001, resulted in a mortality rate of 73%. Only 27% of the crabs that were "lively" before re-immersion were still "lively" after the rehaul. Although more data need to be collected on the survival rate of this species, this high mortality rate, together with the higher incidence of individuals of *P. anamerae* found to be "limp" during vitality assessments (Table 8; 10% compared to 3% of *P. spinosissima* and 1% of *P. formosa*), seems to indicate that this bycatch species might be particularly vulnerable to onboard handling and discarding.

Crabs that were physically damaged (i.e. had missing legs or cracked carapaces) before being subjected to re-immersion were less likely to survive than undamaged crabs. Of the 19 damaged *P. spinosissima* (13 of these were "lively" and 6 were "limp"), 58% did not survive re-immersion and only 32% of these were still "lively." The effect of damage

Table 8

Results of survival-rate experiments. For each experiment the number of re-immersions is given, together with the total number of crabs in lively and limp condition that were re-immersed and their condition on rehauling the crab pots after re-immersion.

	Number of re-immersions	Number of crabs	Initial condition	Condition after re-immersion		
P. formosa						
Experiment 1				Lively	Limp	Dead
(cruise G1)	2	30	Lively	20	6	4
		0	Limp	0	0	0
Experiment 2				Lively	Limp	Dead
(cruise G2)	6	98 (93%)	Lively	81	11	6
		7	Limp	4	1	2
Experiment 3				Lively	Limp	Dead
(cruise H)	3	49 (91%)	Lively	27	21	1
		5	Limp	0	2	3
P. spinosissima						
Experiment 1				Lively	Limp	Dead
(cruise G1)	2	42	Lively	35	3	4
		0	Limp	0	0	0
Experiment 2				Lively	Limp	Dead
(cruise G2)	6	60 (100%)	Lively	55	5	0
			0Limp	0	0	0
Experiment 3				Lively	Limp	Dead
(cruise H)	10	167 (88%)	Lively	71	67	29
		23	Limp	1	6	16

Table 9

Estimation of total survival rate for *P. formosa* and *P. spinosissima* based on results from re-immersion experiments. Because there was no significant difference between the responses of lively animals between experiments 1 and 2, these data were pooled to give a single estimate for the *Argos Georgia*. On the *Argos Georgia* no limp *P. spinosissima* were encountered during the re-immersion experiments; therefore *p(limp,lively)* was set equal to *p(limp,lively)* from experiment 3. Three calculations are presented: the first according to the text description without the rehaul correction, the second including this rehaul, and the third where the original proportions of lively animals $p(lively_o)$ were used from the experimental data in Table 8 rather than the data from the larger sample in Table 6.

	$p(lively_o)$ from Table 6, without rehaul correction	$p(lively_o)$ from Table 6, with rehaul correction	$p(lively_o)$ from Table 8, without rehaul correction	$p(lively_o)$ from Table 8, with rehaul correction
P. formosa				
Argos Georgia	78.7%	79.8%	77.5%	83.7%
Argos Helena	53.7%	56.2%	50.0%	58.4%
P. spinosissima				
Argos Georgia	86.9%	88.5%	88.2%	88.2%
Argos Helena	40.5%	45.4%	37.9%	48.5%

was not so pronounced for *P. formosa*, although of the 12 crabs that were damaged prior to re-immersion (10 were "lively" and 2 were "limp"), only 58% were "lively" upon recovery. The mortality rate of 17% for damaged specimens was more than double the 8% overall mortality found during re-immersion experiments for this species. Most of the dead crabs examined after re-immersion had been attacked by isopods and amphipods and only the shell remained. It is possible that these organisms were in fact responsible for killing the crabs, particularly where damage to the shell allowed access to the softer tissues of the crab.

Calculations of survival rate are given in Table 9 both with and without the re-haul correction. As discussed above, the more accurate estimate of $lively_o$ is probably from Table 6 because of the additional handling stress associated with the experiment. However, Table 9 also presents results obtained from data in Table 8 to estimate this probability. The results suggest that the survival rate of discarded crabs would be high on the *Argos Georgia*, between 77% and 88% for both *P. formosa* and *P. spinosissima* (77–84% for the former, 87–88% for the latter). On the *Argos Helena* discard survival rate was much lower, 50–58% for *P. formosa* and 38–49% for *P. spinosissima*.

Discussion

Crab species

Three previously unreported or rarely reported crab species, *Paralomis anamerae*, *Neolithodes diomedeae* (Benedict, 1894), and *Lithodes murrayi* (Henderson, 1888), were found in our study. The most abundant of these was *P. anamerae*, found at mid-range depths. The only other record of *P. anamerae* is the original description of the species based on four specimens obtained from the continental shelf of Argentina at depths of 132–135 m (Macpherson, 1988). The specimens obtained from South Georgia, at depths of 530–1210 m, therefore represent a considerable southerly extension in the reported geographic distribution of this species, as well as a notable increase in its bathymetric range. López Abellán and Balguerias (1994) reported both *P. spinosissima* and *P. formosa* from a 1986–87 trawl survey on the shelf, but no other species of crabs.

A certain amount of confusion surrounds the identification of *Paralomis* species around South Georgia. *Paralomis aculeata* is found in the CCAMLR database, but almost certainly because of its inclusion in the FAO species identification guide for the Southern Ocean (Arnaud 1985, in Fischer and Hureau, 1985), attributed to Henderson (1888). This species is not mentioned in Macpherson (1988), even as a junior synonym. Conversely, none of the *Paralomis* species identified in the present paper appear in Fischer and Hureau (1985). It is not clear, therefore, which species of *Paralomis* CCAMLR scientific observers have been identifying as *P. aculeata*. Twenty-two specimens of *N. diomedeae* were collected at depths ranging from 420 to 1294 m. This species has previously been recorded from South Georgia (Macpherson, 1988).

Sixteen *L. murrayi* specimens were found on southeast Shag Rocks and west South Georgia, approximately 60 nmi apart, at depths of between 450 m and 605 m. *Lithodes. murrayi* is mainly reported from the southern Indian Ocean around Prince Edward, Crozet, and Possession Islands, as well as Macquarie Islands, Mozambique Channel, and southern New Zealand at depths of 35–200 m (Hale, 1941; Yaldwyn and Dawson, 1970; Arnauld, 1976; Arnauld and Do-Chi, 1977; Kensley, 1977). However, it has been reported in small numbers in CCAMLR statistical catch records from 1993–94, 1997–98 and 1998–99 and by CCAMLR observers (CCAMLR[1]). We have confirmed the identification and the extension of the range of this species to South Georgia. Klages et al. (1995) reported on the distribution of *L. murrayi* off Peter Island, close to the Antarctic continent between 180 and 260 m depth, and a circum-Antarctic distribution has been claimed for the species (Macpherson, 1988). The present study therefore represents the greatest depth recorded for it.

Distribution

Catch rates of *P. spinosissima* encountered in our study were lower than experienced in the September 1995–January 1996 crab fishery by the FV *American Champion*. Watters (1997) reported that average catch rates of legal-size male *P. spinosissima* were between 14.2 and 28.4 males per pot in the Shag Rocks and northwest South Georgia areas (53.5–54°S, 37–40°W). Although the toothfish pots operated by the *Argos Georgia* from March and May 2000 produced catch rates between 0.5 and 4 crabs/pot, the proportion of legal-size males was very low (3%), resulting in legal (retained) crab catch rates of less than 1/pot (note that the retention rates given in Table 2 are lower than those calculated from the length-frequency sampling, Fig. 6). Retention rates for the 1992 FV *Pro Surveyor* cruise (July–August 1992) were 36% (Otto and MacIntosh[5]). The retention rates on the Argos Helena's experimental crab fishery in 1999 were much lower than this (8% and 14% for *P. spinosissima* and *P. formosa* respectively) (Purves[6]). The low retention rate is most likely to be a consequence of the pot design used on vessels G and H, where the collapsible funnel entrances might have restricted the catch to smaller size crabs.

A further feature of the *American Champion* crab fishery was the restriction of fising effort to depths of less than 500 m. The present trials were targeted at toothfish rather than crabs and were conducted according to an experimental plan that distributed fishing effort over time, area, and the full depth range of longlines used in the main toothfish fishery. Accordingly, fishing occurred over a much wider depth range than was used by the previous crab fisheries. Our very high catch rates of *P. formosa* in deep water were therefore not reported by Watters (1997). However, even our catch rates did not result in high numbers of retained legal-size crabs on the *Argos Georgia* because the proportion legally permitted was only 10.5%. Interestingly, even in shallow water (400–800 m) *P. formosa* appeared to be more common than *P. spinosissima*. Only in waters less than 400 m deep did *P. spinosissima* become the dominant species. This finding confirms the results of Watters (1997) who found that *P. spinosissima* catch rates declined at depths deeper than 300 m. Catch rates of *P. spinosissima* were low even in these depths (5 crabs/pot). Only 9 of the total of 110 sets of the Argos Georgia were conducted in depths shallower than 400 m because the main target of the fishery was toothfish.

The differences found in our study in the distribution by depth of the different sexes and sizes of crabs might indicate that recruitment of *P. formosa* takes place in deeper water. This conclusion is based on a decrease in size with increasing depths, the higher proportion of females encountered in deeper water, and increasing crab densities at depth. For *P. spinosissima* this trend was not so pronounced, although crabs of this species were also generally of a smaller size in deeper water. However, contrary to the case with *P. formosa*, females were more prevalent in shallower water. Very few females of *P. spinosissima* were encountered in deep water. These rather unusual findings might suggest ecological partitioning of the benthic habitat, and warrant further investigation.

---

[5] Otto, R. S., and R. A. MacIntosh. 1992. A preliminary report on research conducted during experimental crab fishing in the Antarctic during 1992 (CCAMLR Area 48). Document WG-FSA-92/29, 20 p. CCAMLR, P.O. Box 213, North Hobart, Tasmania, Australia.

[6] Purves, M. G. 1999. Report of the South African designated CCAMLR observer on board the British registered longliner "Argos Helena" in Statistical Subarea 48.3, 31 August to 23 September 1999, 13 p. CCAMLR, P.O. Box 213, North Hobart, Tasmania, Australia.

Another unexpected result of our work was the discovery of a third species of *Paralomis, P. anamerae*, at intermediate depths. This species was apparently not present in the *American Champion* or *Pro Surveyor* catches, presumably again because of the depth restriction in these cruises.

Maturity

For *P. spinosissima* our estimate of 67.3 mm carapace length at 50% male maturity is similar to the 66.4 mm carapace length found by Otto and MacIntosh (1996) at Shag Rocks for this species. Unfortunately, as can be seen from Figure 8, relatively few small *P. formosa* males were encountered and size at maturity for this species (64.0 mm carapace length) is likely to have been poorly estimated in our analysis. However, if it is assumed that male *P. formosa* mature at the same size in relation to female *P. formosa*, as in the case of *P. spinosissima*, the female maturity data presented in Table 5 would suggest that male *P. formosa* would mature at 60.4 mm carapace length (64.3 mm carapace width) rather than the 64.0 mm shown in Figure 8.

Watters and Hobday (1998) have also examined size at maturity for *P. spinosissima* and *P. formosa*, although their samples were taken from South Georgia rather than Shag Rocks. Using a method based on finding the maximum of the second derivative of smoothing spline fits to chela height and carapace width data, they found that size at morphometric maturity for *P. spinosissima* was 73 mm carapace length. This size is similar to that which Otto and Macintosh (1996) obtained for *P. spinosissima* at South Georgia using the same technique as we did. Watters and Hobday's (1998) results for *P. formosa* are, however, for a higher size at maturity (80 mm carapace length) than that for *P. spinosissima*, which would seem to be at variance with our results and the apparent relative sizes of the two species (see Fig. 6 and CCAMLR[2]).

The minimum size limits for crabs at South Georgia were set by CCAMLR in 1992 but, in common with many crab stocks (Schmidt and Pengilly, 1993), these measures were not accompanied by rigorous analysis of the effectiveness of these measures in meeting management objectives. For *P. spinosissima*, Otto and Macintosh's[5] male maturity results for *P. spinosissima* were used, and allowing males at least one opportunity to breed and an assumed growth per moult of 15%, minimum size limits were calculated as 94 mm and 84 mm carapace width at South Georgia and Shag Rocks, respectively (CCAMLR[2]). These results are very similar to our own, but the CCAMLR limit of 102 mm width was based on the then-existing processing requirements rather than on these calculations. Our results suggest, allowing at least one opportunity to breed, that the limit should be 83 mm for *P. spinosissima*. For *P. formosa*, taking our more conservative figure of 64.0 mm carapace length at 50% maturity, the catch size limit should be set at 78 mm carapace width (the less conservative figure, 60.4 mm carapace length, would suggest a size limit of 74 mm carapace width).

Hoggarth (1991) reviewed minimum size limits for a number of stocks of lithodid crabs and found that minimum legal sizes were about 70% of the maximum size for males, which would suggest 85 mm and 84 mm carapace width for *P. spinosissima* and *P. formosa*, respectively. It should, however, also be taken into account that these estimates were probably biased because of the greater sampling effort made at Shag Rocks. Note that the length-frequency distribution for *P. formosa* in Figure 6B appears to indicate a lower maximum size for males of this species than for males of *P. spinosissima*. However, the largest *P. formosa* actually encountered was 120 mm carapace width. Furthermore, Figure 6B seems to be truncated at the larger sizes, suggesting perhaps that a proportion of the large adult population was not encountered during fishing.

Discard mortality

Our results demonstrate that, although a high proportion of crabs is likely to survive the physical strain of being hauled to the surface from potentially great depths, some undersize individuals and nontarget females can be expected to die following discarding. The most significant factor affecting discard survivorship was handling on board the vessel. On the *Argos Georgia,* where crabs were unloaded from pots and sorted on a conveyor belt, survivorships were high, up to 88%, and *P. spinosissima* survived better than *P. formosa.* By contrast, on the *Argos Helena*, where crabs went down a chute prior to processing, survival rate was between 38% and 58% and *P. formosa* survived considerably better than *P. spinosissima*. In general, *P. anamerae* was the most vulnerable species, followed by *P. spinosissima*, and the least vulnerable—*P. formosa*.

Studies of the discard mortality of lithodid crabs in North Pacific fisheries have produced a variety of results. Stevens (1990) found that crabs discarded from commercial sole trawls suffered high mortalities (47.3%), but Byersdorfer and Watson[7] and Zhou and Shirley (1995) both reported relatively low mortalities (<2%) resulting from handling when fishing with pots. Our results support these previous studies and extend them to the Antarctic, clearly indicating that where handling on a pot vessel is reduced, mortalities are relatively low (<15% mortality). When crabs are handled on a pot vessel, as they would be on a trawl vessel, mortalities are higher.

---

[7] Byersdorfer, S., and L. J. Watson. 1992. A summary of biological data collected during the 1991 Bristol Bay red king crab tagging study. Technical Fishery Report 92-14, 30 p. Alaska Department of Fish and Game, Division of Commercial Fisheries, P.O. Box 25526, Juneau, AK 99802-5526.

Other factors, which could not be tested in the re-immersion experiment, may also affect the rate of crab survival. We re-immersed crabs in pots, whereas normally they would be simply dropped into the sea and would be subject to predation from birds and fish before they reached the bottom. The effect of discarding crabs away from their original habitat is unknown, but our results demonstrate a clear depth separation between the two species; therefore one would expect at least an energetic cost if crabs have to relocate. The crabs subjected to re-immersion experiments were sampled immediately before being discarded. They might suffer further damage through the actual discard process; for instance Stevens (1990) speculated that, while traveling through offal chutes, they could become entangled in machinery or suffer further damage upon impact with the surface of the water. Ideally crabs should have been sampled after being through the full discarding procedure, but this was not practical. Finally, eggs often became dislodged during handling and this loss possibly impacted reproductive success.

Zhou and Shirley (1995) presented results that indicate that there are no long-term effects of handling on crab survival, feeding rate, or crab condition; therefore we might reasonably expect that the survival rates seen in our experiments would also be the relevant long-term survival rates. However, even with relatively low discard mortality, the impacts of repeated catching and discarding of individuals will have a cumulative effect on crab populations. Both retained and discarded bycatch should therefore continue to be reported and be incorporated into crab population models. The presence of such a large discarded bycatch might provide the opportunity for the retention (and removal from the population) of parasitized crabs, as suggested by Basson (1994).

Crabs are an inconvenience in a fishery targeting toothfish. *In situ* observations made during the AUDOS experiments on the UK's January 2000 survey confirm that toothfish seek to avoid direct contact with crabs (Yau et al., 2002), although crabs do form a component of their food (Pilling et al., 2001). An inverse relationship was found in the present study between toothfish numbers in pots and crab numbers in pots, suggesting toothfish avoid pots with large crab populations (Fig. 9). Therefore, conducting the toothfish pot fishery in an area of low crab abundance is sensible, and our data do suggest that, at intermediate depths, the crab catch should be low and composed primarily of large *P. formosa*. Avoidance of areas of high crab bycatch will also reduce the mortality associated with discarding female and undersize male crabs. These discard levels are very high (>93%)—considerably higher than those in the Bering Sea (85%: Stevens, 1996). Such high discard levels could be reduced further by developing new pot designs to limit crab catches to larger, legal-size animals—for instance designs with excluder panels (Stevens, 1996)—or perhaps by reducing the minimum size limit.

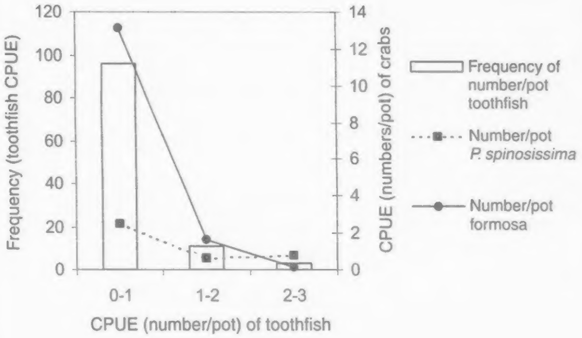


Figure 9

The frequency distribution of the number of toothfish caught per pot compared with the CPUE's of *Paralomis spinosissima* and *P. formosa* (numbers/pot) and of toothfish (*Dissostichus eleginoides*) catches (numbers/pot), as found during the first cruise of the *Argos Georgia* during March to May 2000.

Acknowledgments

We wish to acknowledge the assistance of Argos Ltd. and the excellent cooperation of the captains of the two vessels, Captain Joaquín Abraldes Gonzalez and Captain Jose Andres Sampedro and their crew. Permission to publish these data was kindly granted by Argos Ltd. The Government of South Georgia and the South Sandwich Islands funded the research by MRAG Ltd. on South Georgia fisheries.

Literature citations

Agnew, D. J., T. M. Daw, M. G. Purves, and G. M. Pilling.
2001. Fishing for toothfish using pots: results of trials undertaken around South Georgia, March–May 2000. CCAMLR (Commission for the Conservation of Antartic Marine Living Resources) Sci. 8:93–105.

Agnew, D. J., L. Heaps, C. Jones, A. Watson, K. Berkieta, and J. Pearce.
1999. Depth distribution and spawning pattern of *Dissostichus eleginoides* at South Georgia. CCAMLR Sci. 6:19–36.

Arnauld, P. M.
1976. Peches experimentales de *Lithodes murrayi* Henderson, 1888 (Crustacea, Anomura) aux îles Crozet (SW de l'Océan Indien). Thetys 3:167–172.

Arnauld, P. M., and T. Do-Chi.
1977. Données biologiques et biometriques sur les lithodes *Lithodes murrayi* (Crustacea: Decapoda: Anomura) des Îles Crozet (SW de l'Océan Indien). Mar. Biol. 39:147–159.

Basson, M.
1994. A preliminary investigation of the possible effects of rhizocephalan parasitism on the management of the crab fishery around South Georgia. CCAMLR Sci. 1:175–192.

Benedict, J. E.
1894. Descriptions of new genera and species of crabs of the family Lithodidae, with notes on the young of *Lithodes*

camtschaticus and *Lithodes brevipes*. Proc. U.S. Natl. Mus. 17:479–488.

Fischer, W., and J. C. Hureau (eds.)
1985. FAO species identification sheets for fishery purposes, Southern Ocean, vol. 1., p. 232. FAO (Food and Agriculture Organization of the United Nations), Rome, Italy.

Hale, H. M.
1941. Decapod crustacea. Br. Aust. N.Z. Antarct. Res. Exped. 1929–1931. 48:257–285.

Henderson, J. R.
1888. Report on the Anomura collected by H.M.S. Challenger during the years 1873–76. Rep. Sci. Res. Voy. H.M.S. Challenger, Zool. 27(I-vii):1–221, plates 1–21.

Hoggarth, D. D.
1991. An ecological and economic assessment of the Falkland Islands inshore crab, *Paralomis granulosa*. Ph.D. diss., 312 p. Imperial College, Univ. London, London, UK.

Kensley, B.
1977. The South African Museum's Meiring Naude cruises. Part 2. Crustacea, Decapoda, Anomura and Brachyura. Ann. S. Afr. Mus. 72:161–188.

Klages, M., J. Gutt, A. Starmans, and T. Bruns.
1995. Stone crabs close to the Antarctic continent: *Lithodes murrayi* Henderson, 1888 (Crustacea; Decapoda; Anomura) off Peter I Island (68°51–S, 90°51–W). Polar Biol. 15:73–75.

López-Abellán, L. J., and E. Balguerías.
1994. On the presence of *Paralomis spinosissima* and *Paralomis formosa* in catches taken during the Spanish survey Antartida 8611. CCAMLR Sci. 1:165–173.

Macpherson, E.
1988. Revision of the family Lithodidae Samouelle, 1819 (Crustacea, Decapoda, Anomura) in the Atlantic Ocean. Mongr. Zool. Mar. 2:9–153.

Otto, R. S., and R. A. MacIntosh.
1996. Observations on the biology of the Lithodid crab *Paralomis spinosissima* from the Southern Ocean near South Georgia. *In* Proceedings of the international symposium on biology, management and economics of crabs from high latitude waters, Anchorage, Alaska, October 1995, p. 627–647. Alaska Sea Grant, Fairbanks, AK.

Pilling, G. M., M. G. Purves, T. M. Daw, D. J. Agnew, and J. C. Xavier.
2001. The stomach contents of Patagonian toothfish around South Georgia (South Atlantic). J. Fish Biol. 59: 1370–1384.

Stevens, B. G.
1990. Survival of king and tanner crabs captured by commercial sole trawls. Fish. Bull. 88:731–744.
1996. Crab bycatch in pot fisheries: causes and solutions. *In* Proceedings of the solving bycatch workshop, September 25–27, 1995, Seattle, Washington (T. Wray, ed.), p. 151–158. Alaska Sea Grant, Fairbanks, AK.

Schmidt, D. C., and D. Pengilly.
1993. Review of harvest strategies used in the management of lithodid crab in Alaska. *In* Proceedings of the international symposium on management strategies for exploited fish populations: October 21–24, 1992, Anchorage, Alaska (G. Kruse, D. M. Eggers, R. J. Marasco, C. Pautzke, and T. J. Quinn, eds.), p. 385–407. Alaska Sea Grant, Fairbanks, AK.

Somerton, D. A., and R. S. Otto.
1986. Distribution and reproductive biology of the golden king crab, *Lithodes aequispina*, in the eastern Bering Sea. Fish. Bull. 84:571–584.

Watters, G.
1997. Preliminary analyses of data collected during experimental phases of the 1994/95 and 1995/96 antarctic crab fishing seasons. CCAMLR Sci. 4:141–159.

Watters, G., and A. J. Hobday.
1998. A new method for estimating the morphometric size at maturity of crabs. Can. J. Fish. Aquat. Sci. 55:704–714.

Yaldwyn, J. C., and E. W. Dawson.
1970. The stone crab *Lithodes murrayi* Henderson: the first New Zealand record. Rec. Dom. Mus. (Wellingt.) 6: 275–284.

Yau, C., M. A. Collins, P. M. Bagley, I. Everson, and I. G. Priede.
2002. Scavenging by megabenthos and demersal fish on the South Georgia slope. Antarct. Sci. 14:16–24.

Zhou, S., and T. C. Shirley.
1995. Effects of handling on feeding, activity and survival of red king crabs *Paralithodes camtschaticus* (Tilesius, 1815). J. Shellfish Res. 14:173–177.

The physiological effects of multiple forced submergences in loggerhead sea turtles (*Caretta caretta*)

Erich K. Stabenau
Kimberly R. N. Vietti
Department of Biology
Bradley University
1501 W. Bradley Ave.
Peoria, Illinois 61625
E-mail address (for E. K. Stabenau): eks@bradley.edu

Abstract—Sea turtles are subjected to involuntary submergence and potential mortality due to incidental capture by the commercial shrimp fishing industry. Despite implementation of turtle excluder devices (TEDs) to reduce at-sea mortality, dead stranded turtles continue to be found in near-record numbers along the coasts of the western Atlantic Ocean and northern Gulf of Mexico. Although this mortality may be due to an increase in the number of turtles available to strand, one alternative explanation is that sea turtles are repetitively submerged (as one fishing vessel follows the path of another) in legal TEDs. In the present study, laboratory and field investigations were undertaken to examine the physiological effects of multiple submergence of loggerhead sea turtles (*Caretta caretta*). Turtles in the laboratory study were confined during the submersion episodes, whereas under field conditions, turtles were released directly into TED-equipped commercial fishing nets. Under laboratory and field conditions, pre- and postsubmergence blood samples were collected from turtles submerged three times at 7.5 min per episode with an in-water rest interval of 10, 42, or 180 min between submergences. Analyses of pre- and postsubmergence blood samples revealed that the initial submergence produced a severe and pronounced metabolic and respiratory acidosis in all turtles. Successive submergences produced significant changes in blood pH, P_{CO_2}, and lactate, although the magnitude of the acid-base imbalance was substantially reduced as the number of submergences increased. In addition, increasing the interval between successive submergences permitted greater recovery of blood homeostasis. No turtles died during these studies. Taken together, these data suggest that repetitive submergence of sea turtles in TEDs would not significantly affect their survival potential provided that the animal has an adequate rest interval at the surface between successive submergences.

Manuscript approved for publication 25 March 2003 by Scientific Editor.
Manuscript received 26 June 2003 at NMFS Scientific Publications Office.
Fish. Bull. 101:889–899 (2003).

The five sea turtle species inhabiting the waters of the U.S. Gulf of Mexico and Atlantic Ocean are considered to be threatened or endangered. One contributing factor to sea turtle mortality is incidental capture in the nets of commercial shrimping vessels. The National Research Council's Committee on Sea Turtle Conservation (1990) suggested that as many as 5500 to 55,000 loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempi*) sea turtles were killed annually during shrimping-related activities. More recently, two independent studies statistically confirmed the relationship between shrimping activity and the appearance of stranded sea turtles in the U.S. Gulf of Mexico and the Atlantic Ocean (Caillouet et al., 1991; Crowder et al., 1995). Because of the impact of trawl-related mortality on sea turtle populations, the U.S. government passed regulations in 1987 requiring that commercial shrimping vessels pull nets equipped with certified turtle excluder devices (TEDs). TEDs are designed to exclude any turtle that may enter into shrimping nets, while not affecting the catch of the target species. Crowder et al. (1995) reported that the sea turtle population off the coast of South Carolina continued to decline when TED regulations were implemented; however, the rate of decline decreased significantly after full-time TED use.

In spite of the TED regulations, near-record numbers of dead stranded sea turtles have been found on U.S. Gulf of Mexico and Atlantic Ocean beaches (Shaver-Miller[1]). Although there may be other man-related or natural causes for this continued sea turtle mortality, there are two plausible reasons for the increased mortality during shrimping activities. First, commercial shrimp fishermen generally do not carry legally certified TEDs in their trawl nets and the TEDs that are used are often installed incorrectly or purposely sewn shut. Second, the shrimp fishermen may pull legal TEDs; however, the turtles are repetitively submerged as they are caught in the TEDs of vessels that follow each other. These successive submergences may exacerbate the physiological effects experienced by sea turtles during a forced submersion, and thus, may limit their survival potential.

Sea turtles spend approximately 99% of their time under the surface of the water. During the brief period at the surface, the turtle will exhale and inhale a solitary breath and then dive under the surface (Jackson, 1985). In fact, multiple breaths by sea turtles are generally seen only after prolonged dives. Minimal information is available on the physiological effects of forced submergences of sea turtles. It has been suggested that voluntary dives by sea turtles are aerobic in nature (Wood et al., 1984), whereby oxygen availability minimizes the metabolic production of lactic acid. The turtles may accumulate carbon dioxide, resulting in a respiratory acidosis that is ameliorated by hyperventilation at the surface. Therefore, voluntary diving in the absence of any other external stressor does not limit sea turtle survival potential.

---

[1] Shaver-Miller, D. 2002. Personal commun. Texas coordinator, Sea Turtle Stranding and Salvage Network, USGS, Corpus Christi, Texas 78406.

In contrast, forced submergence of Kemp's ridley and loggerhead sea turtles produces significant blood respiratory and metabolic derangements. Stabenau et al. (1991) reported that forced submergence of Kemp's ridley sea turtles for less than 7.5 min in shrimp nets equipped with TEDs resulted in significant increases in blood lactic acid and Pco_2, and decreases in blood pH. Moreover, several hours were required for these turtles to fully recover blood homeostasis (National Marine Fisheries Service, unpubl. data[2]). However, the study by Stabenau et al. (1991) did not address the physiological effects of multiple forced submergences of sea turtles. It is plausible that repeated submergence induces progressive, significant blood acid-base disturbances, and limits sea turtle survival potential. Therefore, the present study examined the physiological effects of multiple forced submergences on loggerhead sea turtles.

This investigation was divided into two phases. First, a laboratory component was conducted to examine the feasibility of a multiple submergence study. This phase of the research permitted characterization of the magnitude of the acid-base disturbance under controlled conditions. Second, a field investigation was conducted to expose turtles to TED-equipped commercial fishing nets. Data from these studies may offer greater insight into potential sea turtle mortality caused by multiple capture in commercial shrimping nets carrying legal TEDs.

Materials and methods

Laboratory study

Thirty-nine headstarted 2-year-old loggerhead sea turtles reared in captivity at the National Marine Fisheries Service (NMFS) Galveston Laboratory were used in this phase of the study. Each turtle was randomly placed into experimental (submerged, 37.0 ±0.2 cm, 6.51 ±0.06 kg, n=21) or control (nonsubmerged, 36.9 ±0.2 cm, 6.45 ±0.10 kg, n=18) treatments. All turtles were of comparable size and weight and therefore any alterations in blood parameters between experimental and control turtles represented treatment effects rather than size effects. It should be noted that the turtles used in our study were representative of the average size of dead stranded turtles and those animals used in annual TED certification tests.

The study was initiated by collecting presubmergence blood samples from the experimental turtles immediately prior to their individual confinement in a weighted canvas bag. Each turtle was then submerged for 7.5 min in seawater filled tanks. Postsubmergence blood samples were collected within 30 s of bringing the turtle out of the water to minimize blood acid-base changes. Following an in-water rest interval of 10 (treatment 1), 42 (treatment 2), or 180 (treatment 3) min, a presubmergence blood sample was collected and the turtle was submerged a second time. A postsubmergence blood sample was then collected immediately upon surfacing. The turtle was then submerged a third time, following the same rest interval between the first and second submergence episodes, and pre- and postsubmergence blood samples were collected as described above. The seventh serial blood sample was collected 180 min after the final submergence in all turtles. Blood samples were also collected from control turtles over the same time intervals to ensure that repetitive handling and blood sampling did not alter blood homeostasis. All blood samples were collected into heparinized vacutainers from the dorsal cervical sinus as described by Owens and Ruiz (1980). No more than 4–6% of blood volume was collected during the serial sampling to minimize potential physiological effects associated with blood volume depletion.

Field study

Thirty-six headstarted 2-year-old loggerhead sea turtles reared in captivity from the NMFS Galveston Laboratory were used in this phase of the study. The turtles were transported from Galveston, TX, to Panama City, FL, where they were placed into large pens in St. Andrews Bay. The submergence study was initiated after a minimum of 21 days of natural conditioning in the in-water pens. Each turtle was randomly placed into experimental (submerged, 35.9 ±0.2 cm, 6.77 ±0.09 kg, n=24) or control (nonsubmerged, 35.4 ±0.3 cm, 6.46 ±0.12 kg, n=12) treatments. As in the laboratory study, all experimental and control turtles were of comparable size and weight.

The study was initiated by collecting presubmergence blood samples from the experimental turtles immediately prior to their individual confinement in a weighted mesh bag. Each turtle was then submerged using the standard protocol for TED certification tests. Briefly, the mesh bag containing a turtle was placed onto a line connecting the trawl vessel to the headrope on the shrimp net. Divers then released the turtle (without handling the animal) into the mouth of the trawl. Often, turtles were observed vigorously swimming in the trawl until being overcome by the net. Although the shrimp net was equipped with a TED, divers held the escape door closed for 5 min. The turtle was then permitted to leave the trawl and surface. Thus, the total submergence time was approximately 7.5 min, including the time for the weighted mesh bag containing the turtle to reach the headrope for release into the trawl, the 5 min within the trawl, and the time for the turtle to surface. Turtles were immediately captured at the surface and returned to the trawl vessel for postsubmergence blood sampling. Typically, postsubmergence blood samples were collected within 1–2 min of the turtle surfacing. Following a rest interval of 10 (treatment 4), 42 (treatment 5), or 180 (treatment 6) min in water-filled containers on the trawl vessel, a presubmergence blood sample was collected and the turtle was submerged a second time. A postsubmergence blood sample was then collected immediately upon surfacing. The turtle was then submerged a third time, following the same rest interval between the first and second submergence episodes, and pre- and postsubmergence blood samples were collected as described above. A seventh serial blood sample was collected 180 min after the final submergence in all turtles. Blood samples were also collected from nonsubmerged control turtles over the same time intervals to ensure that repetitive handling and blood sampling did not alter blood homeostasis. The blood sampling technique and volume collected was identical to that described for the laboratory component of the study.

---

[2] National Marine Fisheries Service. 1994. Unpubl. data. [Available from E. K. Stabenau, Bradley University, 1501 W. Bradley Ave., Peoria, IL 61625.]

Blood and plasma analyses

In the laboratory study, blood Pco_2 and pH were analyzed immediately following collection by using a clinical blood gas analyzer with electrodes thermostatted at 37°C. Both variables were corrected to turtle cloacal temperature using requisite correction factors for sea turtle blood and plasma (Stabenau and Heming, 1994). In the field study, blood gases (Po_2 and Pco_2) and pH were analyzed on the trawl vessel immediately following collection using a blood gas analyzer with electrodes thermostatted to turtle body temperature (27–28.5°C). The remaining analyses were comparable for both the laboratory and field components of the submergence study. Packed red cell volume (hematocrit) was determined by following centrifugation of heparinized microcapillary tubes. Two hundred microliters of whole blood were then added to 10% trichloroacetic acid for lactate analysis. The deproteinized samples were centrifuged, and the supernatant removed and stored at –70°C. Lactate was determined spectrophotometrically by using standard enzymatic techniques (Sigma, kit 826-B, Saint Louis, MO). The remaining whole blood was then centrifuged, the plasma removed and stored at –70°C. Plasma Na^+ and K^+ were measured with flame photometry (Jenway, model PFP7, Essex, England), and plasma Cl^- was determined with electrometric titration (Haake-Bucher, model 4425000, Saddle Brook, NJ). Plasma glucose was measured spectrophotometrically (Sigma, kit 16-20), and plasma osmolality was determined with a vapor pressure osmometer (Wescor, model 5500, Logan, UT). For the laboratory study, plasma norepinephrine was analyzed with HPLC (BAS, model LC-300, West Lafayette, IN).

All data are expressed as means ±SE. Where appropriate, the data was analyzed with one-way ANOVA. *Post-hoc* comparisons between means were analyzed with Tukey's multiple comparison test. A fiduciary level of $P \le 0.05$ was regarded as significant.

Results

Blood pH, Pco_2, and lactate

The initial submergence of loggerhead sea turtles under laboratory and field conditions produced a dramatic and severe acidosis in all experimental turtles. Blood pH fell an average of 0.54 ±0.03 (range 0.49 to 0.59 pH units) and 0.63 ±0.06 (range 0.53 to 0.73 pH units) in laboratory turtles and field turtles, respectively, following initial submergence (Figs. 1A and 2A). The blood acidosis was derived from respiratory and metabolic components as evident from a positive proton-lactate deficit (Buffer capacity$\times\Delta$pH$-\Delta$[lactate]), and from significant increases in blood Pco_2 and lactate (Figs. 1 and 2). The initial submergence also produced significant decreases in blood Po_2 and increases in plasma norepinephrine ($P \le 0.05$, $n=24$ for Po_2 and $n=11$ for norepinephrine). In contrast, minimal changes in blood pH, Pco_2, and lactate were observed following collection of the first two blood samples in nonsubmerged control turtles (Figs. 1 and 2).

Recovery of the respiratory and metabolic derangements in submerged turtles was dependent on the interval between successive submergences. A 10-min in-water rest interval between the first and second submergence (treatment-1 and -4 turtles) permitted partial recovery of blood pH (Figs. 1A and 2A) and Pco_2 (Figs. 1B and 2B), but blood pH remained significantly different from presubmergence values. Washout of additional lactate was also detected in these animals, whereby blood lactate concentration increased higher than the postsubmergence value (Figs. 1C and 2C). Turtles with a 42-min surface interval (treatment-2 and -5 turtles) between the first and second submergence had partial to complete recovery of blood pH (Figs. 1A and 2A), complete recovery of blood Pco_2 (Figs. 1B and 2B), and slight recovery of blood lactate (Figs. 1C and 2C). Only the blood lactate remained significantly different from the initial presubmergence value after the 42-min rest interval. Turtles with a 180-min in-water recovery interval (treatment-3 and -6 turtles) showed complete recovery of blood pH and Pco_2, although the lactate concentration was slightly higher than baseline levels (Figs. 1 and 2). Blood Po_2 and plasma norepinephrine recovered completely regardless of the surface interval ($P>0.05$, $n=24$ and $n=11$ for Po_2 and norepinephrine, respectively). Nonsubmerged control turtles in the laboratory and the field exhibited few significant changes in blood pH, Pco_2, or lactate, whether the interval between the second and third serial blood sample was 10, 42, or 180 min (Figs. 1 and 2).

The second 7.5-min submergence produced a drop in blood pH and an increase in Pco_2 (Figs. 1 and 2) in all of the experimental animals, and significant differences occurred in treatment 2–6 turtles. It is noteworthy, however, that the severity of the acid-base imbalance was not as drastic as the acidosis measured following the first submergence. The mean pH difference (ΔpH) between the second pre- and postsubmergence ranged from 0.11 and 0.16 in treatment-1 and -4 turtles (animals with a 10-min interval between submergences), respectively, to 0.50 in treatment-3 turtles and 0.66 in treatment-6 turtles (animals with a 180-min interval between submergences). The acidosis in treatment-1 and -4 turtles resulted, in part, from the continual elevation in blood lactate. In contrast, the longer surface interval between the two submergence episodes resulted in enhanced recovery of acid-base variables. Therefore, the turtles with a surface interval of 42 or 180 min had increased production of CO_2 and lactate in relation to turtles with a brief surface interval (Figs. 1 and 2). Comparable changes in blood Po_2 and norepinephrine were measured following the second submergence ($P \le 0.05$, $n=24$ and $n=9$

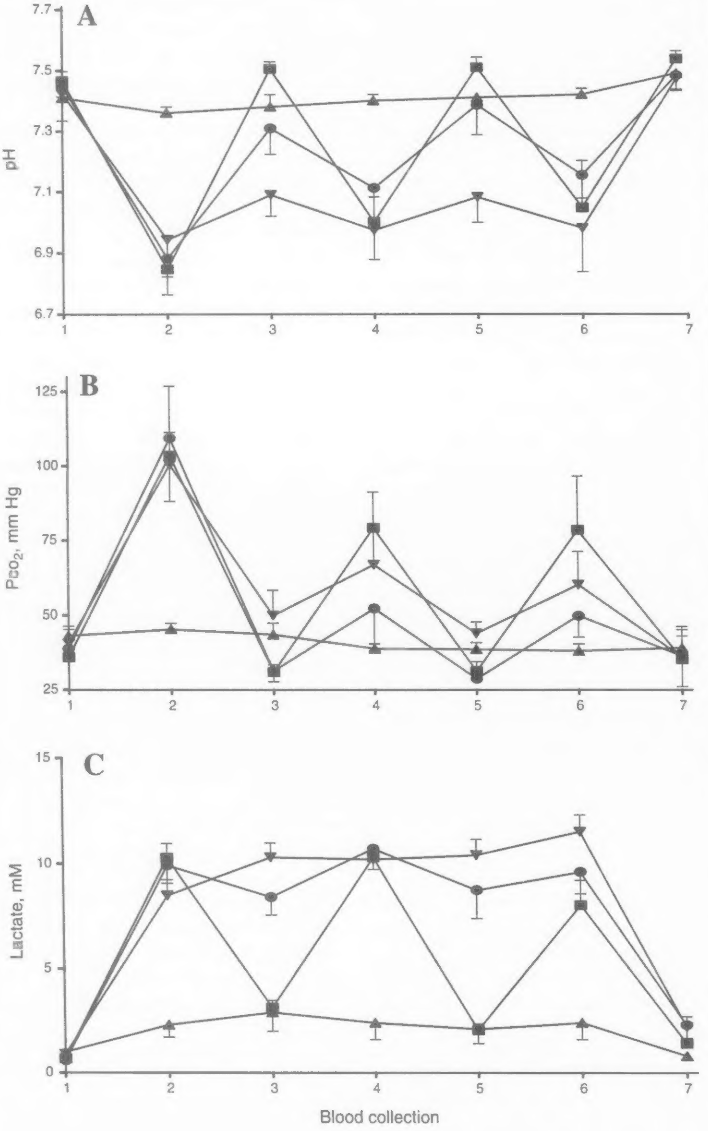


Figure 1

Blood pH (**A**), P_{CO_2} (**B**), and lactate (**C**) measured prior to and after three successive forced submergence episodes in loggerhead sea turtles in the laboratory. Blood collection 1, 3, and 5 are presubmergence samples, whereas blood collection 2, 4, and 6 are postsubmergence samples. Blood collection 7 was taken 180 min after the final submergence. The surface interval between the submergences was 10 min (▼), 42 min (●), or 180 min (■). Data from control sea turtles (▲) are shown for comparison.

for P_{O_2} and norepinephrine, respectively). Collection of the fourth sample from nonsubmerged control turtles revealed no significant changes in blood pH, P_{CO_2}, or lactate when compared to the third sample (Figs. 1 and 2).

The remaining serial blood samples revealed comparable patterns in the blood pH, P_{CO_2}, P_{O_2}, lactate, and norepinephrine. Turtles given a longer rest interval at the surface (after the second submergence) had enhanced recovery of

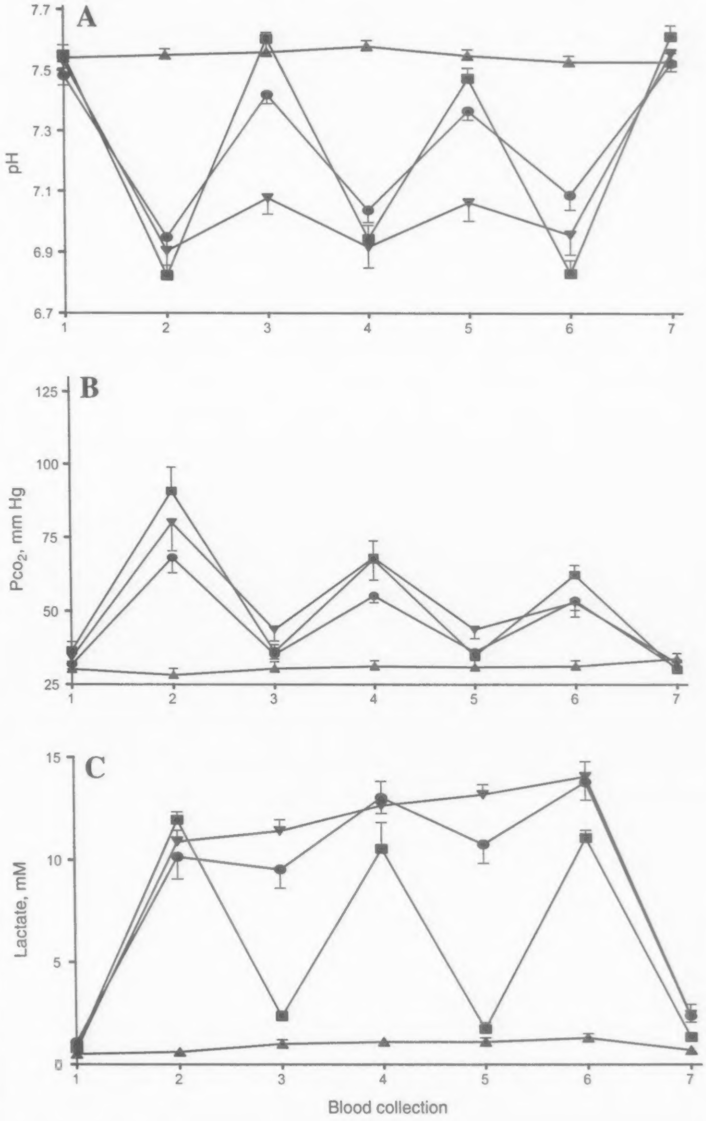


Figure 2

Blood pH (**A**), Pco_2 (**B**), and lactate (**C**) measured prior to and after three successive forced submergence episodes in loggerhead sea turtles in TED-equipped nets. Blood collection 1, 3, and 5 are presubmergence samples, whereas blood collection 2, 4, and 6 are postsubmergence samples. Blood collection 7 was taken 180 min after the final submergence. The surface interval between the submergences was 10 min (▼), 42 min (●), or 180 min (■). Data from control turtles (▲) are shown for comparison.

blood acid-base variables, whereas a brief surface interval permitted minimal recovery of blood homeostasis (Figs. 1 and 2). Submersion of experimental turtles a third time resulted in similar changes in blood pH, Pco_2, and lactate to that measured following the second submersion, and the length of the at-surface rest interval affected the magnitude of recovery of blood acid-base status. The seventh serial sample collected 180 min after the final postsubmer-

Table 1

Mean (±SE) plasma Na^+, K^+, and plasma osmotic pressure (OP) prior to and following laboratory multiple forced submergences of sea turtles with a 10-min, 42-min, or 180-min rest interval. Serial blood sampling regime is described in the "Materials and methods" section. Significant differences between samples 1 and 2, 3 and 4, and 5 and 6 are indicated by an asterisk (*), whereas significant differences of samples from the initial blood sample (serial sample 1) are denoted by a pound sign (#).

	10 min			42 min			180 min		
Treatment	Na^+ (mM)	K^+ (mM)	OP (mosm/kg)	Na^+ (mM)	K^+ (mM)	OP (mosm/kg)	Na^+ (mM)	K^+ (mM)	OP (mosm/kg)
Control	153 ±3	4.0 ±0.2	319 ±3	152 ±4	3.9 ±0.3	305 ±2	158 ±4	4.3 ±0.4	322 ±4
Serial sample									
1	144 ±5	4.5 ±0.3	319 ±6	158 ±6	3.9 ±0.2	314 ±11	162 ±2	4.1 ±0.1	296 ±3
2	159 ±6	5.9 ±0.6	341 ±4	163 ±3	6.1 ±0.6*#	364 ±10*#	187 ±2*#	6.9 ±0.3*#	342 ±5*#
3	145 ±3	4.9 ±0.2	330 ±5	156 ±2	4.1 ±0.3	336 ±8	160 ±4	4.4 ±0.1	308 ±4
4	166 ±7#	6.2 ±0.3#	351 ±14#	160 ±6	5.5 ±0.2#	342 ±15	179 ±4	6.7 ±0.5*#	339 ±4*#
5	158 ±6	5.1 ±0.1	335 ±11	147 ±6	4.1 ±0.3	334 ±12	158 ±6	3.9 ±0.2	305 ±5
6	154 ±5	6.1 ±0.3#	340 ±12	157 ±9	4.8 ±0.3	345 ±12#	181 ±2	5.7 ±0.5*	323 ±8#
7	139 ±3	4.8 ±0.4	323 ±8	149 ±6	4.4 ±0.3	331 ±7	158 ±10	4.4 ±0.5	305 ±3

gence sample revealed that blood pH, Pco_2, and lactate recovered completely for all experimental turtles (Figs. 1 and 2). Minimal changes in blood pH, Pco_2 and lactate were detected in laboratory and field control turtles during collection of the 5–7 serial blood samples (Figs. 1 and 2).

Ions, glucose, and osmotic pressure

Postsubmergence blood samples from laboratory turtles revealed elevations in plasma Na^+, K^+, and osmotic pressure when compared to the corresponding presubmergence values (Table 1). Significant increases in the plasma Na^+, K^+, and osmotic pressure were observed more frequently in turtles with a longer in-water rest interval between successive submergences (Table 1). In contrast, the plasma ion concentrations and osmotic pressure of control turtles did not substantially change ($P>0.05$, $n=9$) during serial blood sample collection. In addition, no significant differences in plasma glucose and Cl^- ($P>0.05$, $n=10$) were measured in any of the experimental turtles. Although most of the postsubmergence changes in the blood parameters in experimental turtles were not significant (Table 1), and minimal alterations in blood chemistry were observed in control turtles, the results suggested that there was a relationship between blood acid-base status and plasma osmolality and ion concentration. Therefore, correlation analyses were used to determine the interdependence of these variables.

Figure 3 shows the results of the correlation analyses, where pH is plotted versus ion concentration (i.e. Na^+, K^+, and Cl^- concentration), osmolality, or hematocrit. Nonsubmerged control turtles had a significant correlation between blood pH and plasma chloride, and pH and hematocrit (Fig. 3). As pH declined, there were slight, yet significant, increases in the $[Cl^-]$ and hematocrit. However, no correlation was detected between pH and plasma $[Na^+]$, $[K^+]$, or osmolality in these animals. In contrast, a significant correlation was detected between blood pH and plasma $[Na^+]$, $[K^+]$, $[Cl^-]$, osmolality, and hematocrit in experimentally submerged turtles (Fig. 3). In each case, a decrease in blood pH led to an increase in the correlated variable. These data are consistent with significant water movement into and out of the red blood cells during and after forced submersion.

Brief forced submergence of loggerhead turtles in trawl-equipped fishing nets had a profound effect on the plasma ionic status (Table 2). Plasma $[K^+]$ increased significantly immediately following submergence in all experimental turtles. Significant increases were also observed in the plasma $[Na^+]$ and osmotic pressure, although these changes did not occur in turtles from all of the experimental treatments (Table 2). Turtles partially to completely recovered from the ionic imbalances, although subsequent submergences caused significant increases in plasma K^+ and nonsignificant increases in plasma Na^+ and osmolality in most experimental turtles (Table 2). Ionic homeostasis in forcibly submerged turtles was achieved within 180 min of the final submergence, whereby plasma ion concentrations were comparable to the initial presubmergence values (Table 2). The plasma ion concentrations and osmotic pressure in nonsubmerged control turtles were unaffected by serial blood sampling. Thus, ionic changes in experimental turtles resulted from the forced submergence and not from handling and repetitive blood sampling.

Discussion

Acid-Base status

Multiple submergences of 2-year-old loggerhead sea turtles under laboratory and field conditions produced sig-

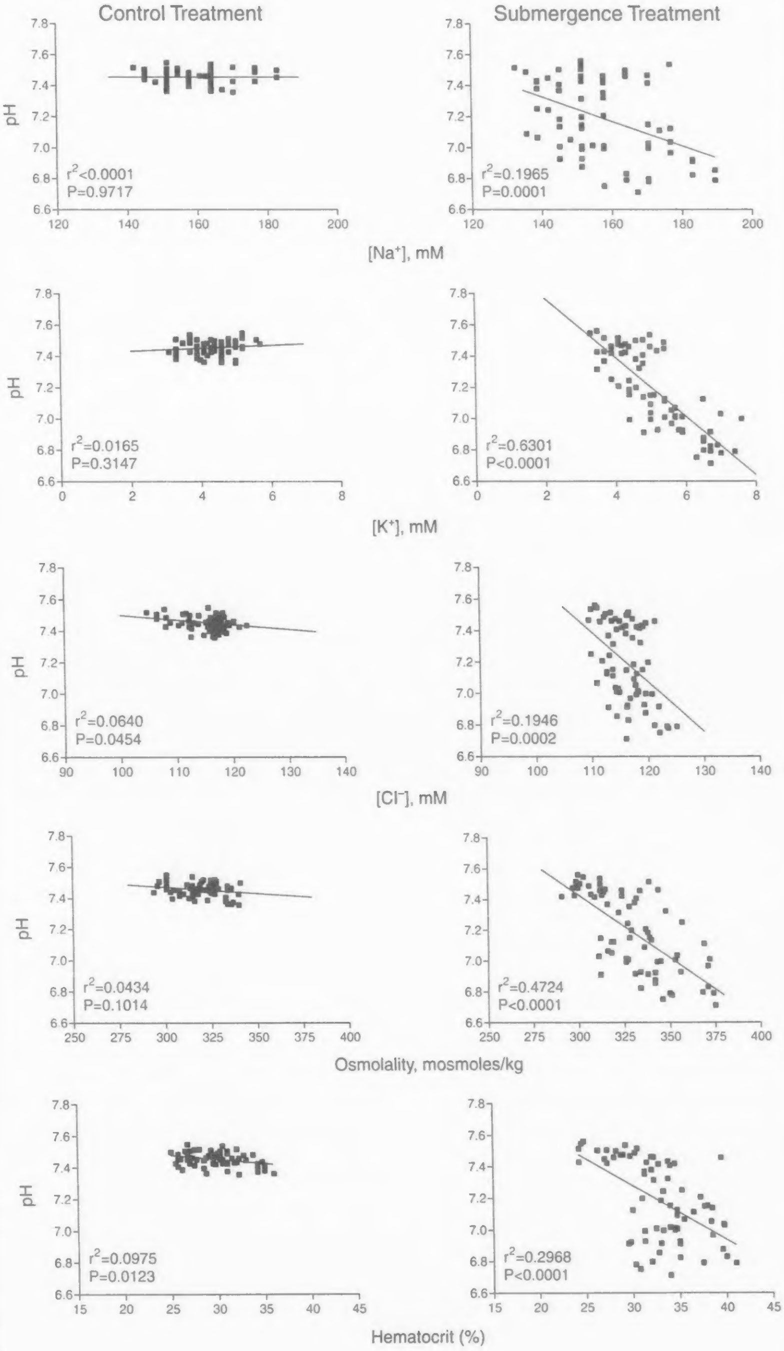


Figure 3

Relationship between blood pH and plasma [Na⁺], [K⁺], [Cl⁻], osmolality, and hematocrit in control (left column) and submerged (right column) loggerhead sea turtles. The lines are best fits to the data. Significance of the correlated variables is noted on each figure.

Table 2

Mean (±SE) plasma Na$^+$, K$^+$, and plasma osmotic pressure (OP) prior to and following multiple forced submergences of sea turtles in TED-equipped nets with a 10-min, 42-min, or 180-min rest interval. Serial blood sampling regime is described in the "Materials and methods" section. Significant differences between samples 1 and 2, 3 and 4, and 5 and 6 are indicated by an asterisk (*), whereas significant differences of samples from the initial blood sample (serial sample 1) are denoted by a pound sign (#).

	10 min			42 min			180 min		
Treatment	Na$^+$ (mM)	K$^+$ (mM)	OP (mosm/kg)	Na$^+$ (mM)	K$^+$ (mM)	OP (mosm/kg)	Na$^+$ (mM)	K$^+$ (mM)	OP (mosm/kg)
Control	150 ±3	3.0 ±0.2	313 ±8	139 ±6	3.4 ±0.2	321 ±7	151 ±1	3.1 ±0.1	310 ±5
Serial sample									
1	153 ±2	3.3 ±0.3	318 ±4	160 ±4	3.1 ±0.2	331 ±12	164 ±2	4.5 ±0.7	325 ±9
2	171 ±8	5.5 ±0.3$^{*\#}$	345 ±4$^{*\#}$	186 ±8$^{*\#}$	5.0 ±0.4$^{*\#}$	368 ±10	188 ±4	7.0 ±0.6$^{*\#}$	355 ±3$^{*\#}$
3	156 ±6	4.3 ±0.0$^{\#}$	332 ±4	163 ±3	2.8 ±0.1	338 ±11	163 ±10	3.6 ±0.3	314 ±3
4	171 ±8	5.3 ±0.1$^{*\#}$	349 ±1$^{\#}$	181 ±3	4.9 ±0.3$^{*\#}$	361 ±13	176 ±10	6.2 ±0.3$^{*\#}$	352 ±9*
5	166 ±4	4.3 ±0.1$^{\#}$	334 ±2	160 ±8	2.9 ±0.2	332 ±9	173 ±10	4.0 ±0.2	323 ±3
6	166 ±12	5.1 ±0.1$^{\#}$	335 ±11	185 ±4$^{*\#}$	4.5 ±0.4$^{*\#}$	343 ±14	175 ±18	5.3 ±0.0	333 ±11
7	157 ±4	3.7 ±0.1	325 ±2	161 ±6	2.6 ±0.2	326 ±9	159 ±11	3.6 ±0.6	320 ±4

nificant blood metabolic and respiratory disturbances. The most dramatic changes in blood pH, Pco$_2$, and lactate occurred following the first of the three forced submergences in all of the experimental turtles (Table 3). Under laboratory conditions, the turtles exhibited an average pH change of 0.54 U following the first submergence, whereas initial submergence of 2-year-old loggerhead sea turtles in TED-equipped commercial fishing nets induced a pH decrease of 0.63 U. The initial acid-base disturbances measured in our study were comparable in magnitude to those measured in Kemp's ridley and loggerhead sea turtles in standard TED certification trials (Table 3).

The second and third submergences of 2-year-old loggerheads sea turtles did not result in similar changes in blood pH, Pco$_2$, and lactate, as was measured following the initial submergence (Table 3). To our knowledge, no information is available in the literature on the physiological effects of multiple submergences in sea turtles for comparison. Obviously, the interval between the submergence episodes directly influenced the magnitude of the blood acid-base imbalance during successive submergences. A longer time interval at the surface led to enhanced recovery of blood pH, Pco$_2$, and lactate. Lutz and Dunbar-Cooper (1987) reported that loggerhead sea turtles captured during trawling at Cape Canaveral, Florida, exhibited a 16.8% decline in lactate 180 min following submergence. Those authors proposed that the rate of lactate decline was dependent on the magnitude of the lactate concentration, so that 10 mM of lactate would decline at a rate of 1.25 mM lactate/h. However, in the present study, the rate of lactate decline was considerably higher than that suggested by Lutz and Dunbar-Cooper (1987). Lactate declined 70.0% and 79.6% within 180 min of the submergence episodes in treatment 3 turtles, whereas no decline was measured in treatment 1 turtles (10 min interval) between submergences. In fact, it was apparent that lactate continued to washout into the bloodstream during the 10-min recovery phases in these turtles (Fig. 1, Table 3). Thus, turtles with a brief period between the submergence episodes would have a limited ability to release the CO$_2$ retained during submersion or to break down lactic acid produced during the course of the forced dive. Lactate declined 15.2% and 18.7% during the 42-min interval between submergences in treatment-2 turtles. Blood lactate declined 80.9%, 76.0%, and 82.5% in treatment-1, -2, and -3 turtles, respectively, during the final 180-min recovery period. Thus, the overall rate of lactate decline in the final 180 minutes of the laboratory study was 2.6 ±0.2 mM/h. Finally, the elevated lactate concentration in sea turtles during the 180-min postsubmergence recovery time interval suggests that the samples were collected too soon to permit complete recovery of blood lactate.

Comparable rates of lactate clearance measured in the laboratory submergence study were detected following forced submergences of loggerhead sea turtles in TED-equipped fishing nets. Substantial retention of CO$_2$ and additional washout of lactate occurred during the 10-min postsubmergence recovery interval in treatment-4 turtles. Treatment-5 turtles exhibited a 6% drop in the blood lactate concentration during the first 42-min postsubmergence recovery interval and a 17.5% decrease in the blood lactate during the second recovery interval. Thus, the 42-min postsubmersion recovery interval permitted recovery of blood gases, but was inadequate to clear the blood lactate (Fig. 2, Table 3). Lactate declined 80.4% and 83.8%, respectively, during the first two 180-min postsubmergence recovery intervals in treatment-6 turtles. As was the case for laboratory submerged sea turtles, a longer surface interval ultimately resulted in an increased ability to recover from the submersion episodes. In fact, lactate declined 82.7%, 82.8%, and 87.9%, respectively, in treatment-4, -5, and -6 turtles 180 minutes after the final submersion episode (Fig. 2, Table 3).

Table 3

Effects of forced submergence on blood pH, Pco_2, and lactate in Kemp's ridley (LK) and loggerhead (CC) sea turtles. Data are expressed as the mean difference (Δ) between post- and presubmergence values. Data from this study are provided from the three submergence episodes of treatment 1–3 turtles under the laboratory protocol and treatment 4–6 turtles in the field protocol. ND = not determined.

Species	Turtle size (kg)	Submergence duration (min)		ΔpH	$\Delta\mathrm{Pco}_2$ (mm Hg)	Δlactate (mM)	Reference
LK	5–16.5	≤7.3		0.37	12.8	8.5	Stabenau et al. (1991)
	5–6	≤7.3		0.31	24.5	15.1	TED certification tests[1]
CC	5–6	4.3		0.33	ND	13.4	TED certification tests[1]
	5–6	12.5		0.52	ND	17.2	
CC	6.5–7.0	7.5	treatment 1	1) 0.49	61.1	7.6	Laboratory study
				2) 0.11	16.3	–0.1	
				3) 0.10	15.3	1.1	
			treatment 2	1) 0.57	70.8	9.3	
				2) 0.20	20.9	2.3	
				3) 0.23	21.1	1.1	
			treatment 3	1) 0.59	98.7	9.6	
				2) 0.50	68.6	7.2	
				3) 0.46	67.3	5.9	
			treatment 4	1) 0.63	45.8	10.2	Field study
				2) 0.16	24.5	1.9	
				3) 0.11	9.3	0.9	
			treatment 5	1) 0.53	36.3	9.1	
				2) 0.38	19.9	3.5	
				3) 0.28	17.5	3.0	
			treatment 6	1) 0.73	54.2	11.2	
				2) 0.66	31.3	9.2	
				3) 0.65	27.5	9.3	

[1] Data were collected by one of the authors (EKS) during standard TED certification tests in 1993–94. Samples were collected from the cervical sinus of Kemp's ridley and loggerhead sea turtles prior to and following forced submergences in a commercial shrimp net equipped with a TED. Turtles in these studies were permitted to exit the TED-equipped net.

It must be noted that any discussion on lactate production and recovery following submersion is applicable to environmental conditions comparable to those reported in this study. For example, lactate formation and recovery rates of lactate build-up would be significantly influenced by water temperature. Longer recovery rates may take place in cold water, whereas warmer waters may lead to additional lactate production thereby influencing the rate of lactate elimination. In addition, the blood lactate concentrations measured in this study may underestimate the true lactate burden. Lactate has been shown to partition into other tissues, including the shell, following submersion of freshwater turtles (Jackson et al., 1999). Finally, sea turtle size could potentially alter lactate production and elimination. Results from submersion experiments conducted in our laboratory indicate that smaller animals exhibit a significant acidosis and lactate build-up in comparison to larger sea turtles. Whether less acidosis and lactate build-up is due to additional lactate buffering by the larger sea turtles warrants further investigation.

Ions, osmolality, and hematocrit

There are three primary mechanisms for recovery of blood pH following an acid-base disturbance: cellular buffering, and respiratory and renal compensation. Cellular responses occur immediately following the disturbance, whereas respiratory and renal adjustments occur within minutes to hours, respectively. Previously, Stabenau et al. (1991) reported that Kemp's ridley sea turtles exhibited a significant increase in plasma [K$^+$] following trawl submergences. However, those authors reported that trawl stress had no effect on plasma [Cl$^-$], [Na$^+$], or hematocrit. In the present study, a cellular response to the severe acid-base disturbance caused by the multiple forced submergences was suggested by alterations in plasma ion concentrations, osmolality, and hematocrit during the blood acidosis. As shown in Figure 3, decreases in blood pH were correlated with increases in [K$^+$], [Na$^+$], [Cl$^-$], osmolality, and hematocrit.

Hematocrit (percent packed red blood cells) changes may result from washout of additional red blood cells into the bloodstream, from areas such as the spleen, in order to provide more red blood cells during the hypoxic phases of the forced submergence. This explanation, however, is unlikely given that substantial fluctuations in hematocrit were observed during the course of the submergence experiments and that a normal hematocrit was measured in the final serial blood sample. A more plausible explanation is that there was an osmotically obliged influx of water into the red blood cells, swelling the cells, and leading to increases in hematocrit, and in plasma ion concentration and osmotic pressure. Red cell volume is regulated in animals through transport of intracellular and extracellular solutes. Although there is minimal information available in the literature concerning regulatory volume transport in reptiles, the mechanisms of regulatory volume increase (RVI) and regulatory volume decrease (RVD) are known in other lower vertebrates. For example, Cala (1983) reported that in *Amphiuma* (amphiuma [common name]) red cells, the mechanism of RVD is K^+_{out}/H^+_{in} counter-transport coupled with $Cl^-_{out}/HCO_3^-{}_{in}$ exchange (where the subscripts in and out represent transport into and out of the cell, respectively), whereas RVI is accomplished by Na^+_{in}/H^+_{out} transport coupled with $Cl^-_{in}/HCO_3^-{}_{out}$ exchange (Cala, 1983). Other studies have suggested that red cell RVD occurs because of electroneutral KCl cotransport out of the cell and RVI occurs because of electroneutral NaK2Cl or NaCl cotransport into the cell (Haussinger and Lang, 1991). It is impossible to determine which of these mechanisms, if any, were involved in regulating red cell volume in sea turtles during and following forced submergence. These transporters, however, have been shown to be sensitive to cellular hypoxia (i.e. low Po_2) and low blood pH (Cossins and Gibson, 1997)—conditions present in the experimental turtles following submergence. In addition, hypoxic and acidotic conditions were absent in nonsubmerged control turtles which did not experience substantial shifts in plasma ion concentrations, osmotic pressure, or hematocrit.

Effects of handling

Significant changes in blood pH, Pco_2, and lactate were occasionally detected in nonsubmerged control turtles. However, it is impossible to determine if these changes resulted from repetitive handling during blood sampling or from increased activity while free-swimming in a large circular tank following blood collection. Nevertheless, control turtle blood lactate concentration was substantially less than the lactate measured following forced submergence in experimental turtles (Figs. 1 and 2). In addition, the blood pH remained fairly constant in the control turtles during collection of the seven serial samples.

Laboratory versus field experimentation

It should be noted that conducting the study under laboratory and field conditions provided unique benefits for analyzing the physiological effects of submersion. For example, the laboratory conditions permitted collection of blood samples immediately upon termination of the submersion period, whereas in the field, sea turtles had to be transported back to the trawl vessel for postsubmersion blood sampling. Turtles forcibly submerged under laboratory or field conditions hyperventilated upon surfacing. Stabenau et al. (1991) reported a 9- to 10-fold increase in the breathing frequency of trawled Kemp's ridley sea turtles. Comparable breathing rates were observed in the present study after submersion and, thus, it is plausible that the blood Pco_2 measured in turtles under field conditions underestimated the actual buildup in blood CO_2 (see Table 3 for a comparison of the blood Pco_2 under laboratory and field conditions). In contrast, the field experiment permitted examining the physiological stress of semiwild turtles in TED-equipped commercial fishing nets following a minimum of 21 days of in-water conditioning. The greater acidosis measured in forcibly submerged turtles resulted from increased swimming activity during the forced submergence. This is confirmed by a postsubmergence increase in blood lactate of 10.1 mM under trawling conditions versus 8.8 mM following laboratory submergence. Under laboratory and field conditions, the behavior of the turtles following submergence was monitored up to their release. It is unclear, however, if the acid-base and ionic imbalance caused by forced submersions would alter long-term normal physiology and behavior. It is plausible that repetitive alteration of blood pH by the magnitude measured in the present study may have pathological consequences. For example, no information is available on whether turtles resume normal diving and feeding behavior following prolonged or multiple forced submersions, or whether turtles become more susceptible to repeated submersions in TED-equipped nets.

Use of turtles reared in captivity

Two-year-old loggerhead sea turtles reared in captivity were used for all of the submergence experiments. It was assumed that these animals were adequate surrogates for wild sea turtles. In fact, similar-size animals from the NMFS Galveston Laboratory are used in annual TED certification trials. Nevertheless, there may be differences in the physiology of captive and wild turtles subjected to forced submergences. For example, it is possible that wild sea turtles would be exposed to forced submergences following lengthy, voluntary dives. No information is available in the literature on the acid-base and ionic status of wild sea turtles following prolonged voluntary dives or forced multiple submergences. If dives are anaerobic, then subjecting wild sea turtles to multiple forced submergences may adversely affect survival potential.

Conclusions

The data suggest that forced submergences of 2-year-old loggerhead sea turtles reared in captivity produce significant blood metabolic and respiratory acidosis. Repetitive submergences did not augment the acidosis, rather subsequent submergences resulted in less severe acid-base disturbances. Under trawl conditions, the turtle must recover from any physiological acid-base disturbance when it is freed from a TED-equipped net. Recovery is accomplished, in part, by the turtle immediately surfacing and hyperventilating (Jackson, 1985; Stabenau et al., 1991). This behavior was observed following each submergence episode. Turtles would then resume normal voluntary diving behavior, presumably after partial-to-complete recovery from the acid-base disturbance. These data suggest that repetitive submergences of sea turtles in TED-equipped nets would not significantly affect their survival potential, provided that the turtles have a recovery interval between successive submergences. However, it should be noted that the latter statement is based on comparable-size turtles that may be submerged in shrimp nets equipped with legally certified and installed turtle excluder devices. Poor installation or lack of use of legal TEDs would result in augmenting the acid-base imbalance in the turtles. Increasing the magnitude of the blood acid-base and ionic disturbance during each submersion would increase the length of time necessary to achieve partial or complete recovery.

Acknowledgments

Grateful appreciation is expressed to personnel from the National Marine Fisheries Service Galveston and Pascagoula Laboratories for their assistance in turtle husbandry and in conducting the submersion protocol under laboratory and very difficult field conditions. These studies were conducted under appropriate threatened and endangered species permits issued by the U.S. Fish and Wildlife Service, Texas Park and Wildlife Department, and the Florida Department of Natural Resources.

Literature cited

Caillouet, C. W., M. J. Duronslet, A. M. Landry, D. B. Revera, D. J. Shaver, K. M. Stanley, R. W. Heinly, and E. K. Stabenau.
1991. Sea turtle strandings and shrimp fishing effort in the Northwestern Gulf of Mexico, 1986–89. Fish. Bull. 89:712–718.

Cala, P. M.
1983. Volume regulation by red blood cells: mechanisms of ion transport. Mol. Physiol. 4:33–52.

Cossins, A., and J. Gibson.
1997. Volume-sensitive transport systems and volume homeostasis in vertebrate red blood cells. J. Exp. Biol. 200:343–352.

Crowder, L. B., S. R. Hopkins-Murphy, and J. A. Royle.
1995. Effects of turtle excluder devices (TEDs) on loggerhead sea turtle strandings with implications for conservation. Copeia 1995:773–779.

Haussinger, D., and F. Lang.
1991. The mutual interaction between cell volume and cell function: a new principle of metabolic regulation. Biochem. Cell Biol. 69:1–4.

Jackson, D. C.
1985. Respiration and respiratory control in the green turtle, *Chelonia mydas*. Copeia 1985:664–671.

Jackson, D. C., Z. Goldberger, S. Visuri, and R. N. Armstrong.
1999. Ionic exchanges of turtle shell *in vitro* and their relevance to shell function in the anoxic turtle. J. Exp. Biol. 202(part 5):513–520.

Lutz, P. L., and A. Dunbar-Cooper.
1987. Variations in blood chemistry of the loggerhead sea turtle *Caretta caretta*. Fish. Bull. 85:37–44.

National Research Council.
1990. Decline of sea turtles: causes and prevention, 259 p. National Academy Press, Washington, DC.

Owens D. W., and G. J. Ruiz.
1980. New methods of obtaining blood and cerebrospinal fluid from marine turtles. Herpetologica 36:17–20.

Stabenau, E. K., and T. A. Heming.
1994. The *in vitro* respiratory and acid-base properties of blood and tissue from the Kemp's ridley sea turtle, *Lepidochelys kempi*. Can. J. Zool. 72:1403–1408.

Stabenau, E. K., T. A. Heming, and J. A. Mitchell.
1991. Respiratory, acid-base and ionic status of Kemp's ridley sea turtles (*Lepidochelys kempi*) subjected to trawling. Comp. Biochem. Physiol. 99A:107–111.

Wood, S. C., R. N. Gatz, and M. L. Glass.
1984. Oxygen transport in the green sea turtle. J. Comp. Physiol. B. 154:275–280.

Quantitative determination of the timing of otolith ring formation from marginal increments in four marine teleost species from northwestern Australia

Peter C. Stephenson
Western Australian Marine Research Laboratories
West Coast Drive (off Elvire St)
Waterman, Western Australia, 6020, Australia
E-mail address: pstephenson@fish.wa.gov.au

Norm G. Hall
School of Biological and Environmental Sciences
Murdoch University
Murdoch, Western Australia, 6150, Australia

Abstract—The sectioned otoliths of four fish species from a tropical demersal trawl fishery in Western Australia revealed a series of alternating translucent and opaque zones in reflected light. The translucent zones, referred to as growth rings, were counted to determine fish ages. The width of the opaque zone on the periphery of the otolith section as a proportion of the width of the previous opaque zone (index of completion) was used to determine the periodicity of growth-ring formation.

This article describes a method for modeling changes in the index of ring completion over time, from which a parameter for the most probable time of growth-ring formation (with confidence intervals) can be determined. The parameter estimate for the timing of new growth-ring formation for *Lethrinus* sp. 3 was from mid July to mid September, for *Lutjanus vitta* from early July to the end of August, for *Nemipterus furcosus* from mid July to late September, and for *Lutjanus sebae* from mid July to mid November. The confidence intervals for the timing of formation of growth rings was variable between species, being smallest for *L. vitta*, and variable between fish of the same species with different numbers of growth rings.

The stock assessments of these commercially important species relies on aging information for all the age classes used in the assessment. This study demonstrated that growth rings on sectioned otoliths were laid down annually, irrespective of the number of growth rings, and also demonstrated that the timing of ring formation for these tropical species can be determined quantitatively (with confidence intervals).

Manuscript approved for publication 3 June 2003 by Scientific Editor.

Manuscript received 26 June 2003 at NMFS Scientific Publications Office.

Fish. Bull 101:900–909 (2003).

The Pilbara fish trawl fishery, operating on the North West Shelf of Western Australia, has developed rapidly in the last ten years and is now the most valuable commercial scalefish fishery in Western Australia. Catch from this fishery was valued at $7 million (wholesale value) in 2001. In this multispecies fishery, *Lutjanus vitta* (Quoy and Gaimard, 1824) (brownstripe red snapper), *Nemipterus furcosus* (Valenciennes, 1830) (fork-tailed threadfin bream, also known as rosy threadfin bream), *Lethrinus* sp. 3 (Carpenter and Niem, 2001) (lesser spangled emperor, known locally as blue-spot emperor) made up 8%, 10%, and 20% respectively of the total scalefish trawl catch in 2000. The highly prized species, *Lutjanus sebae* (Cuvier, 1828) (red emperor), although comprising only 4% of the catch, is important because of its high market value.

In 1993 a research project was commenced to determine the fishing effort required for optimal level of catches in the Pilbara trawl fishery (Stephenson and Dunk[1]). The project relied on validated age composition data for *L.vitta*, *L.* sp. 3, *N. furcosus*, and *L. sebae*. The growth rings on otoliths have been shown to be formed annually for only one to three growth rings for *N. furcosus* (Sainsbury and Whitelaw, 1984), and for two to three growth rings for *L. sebae* (McPherson and Squire, 1992). After pooling of all age classes, Davis and West (1992) showed that growth rings of *L. vitta* were formed annually.

Determining age composition involves counting growth rings on hard parts of fish (otoliths, scales, spines, bones) and determining the timing of growth-ring formation. Sagittal otoliths are commonly used for aging teleost fishes and recent studies (Hyndes et al., 1992; Milton et al., 1995; Newman et al., 1996) have indicated that for some species sectioned otoliths give more reliable age estimates than whole, or broken-and-burnt otoliths. The periodicity of ring formation is commonly determined by the mark-recapture method in which fish are injected with chemical markers and the number of rings created between injection and recapture are compared (Ferreira and Russ, 1992; Francis et al., 1992; Newman et al., 1996).

An alternative to mark-recapture is marginal increment analysis in which the distance from the growth ring to the edge of the otolith, for a sample of fish, is tracked over time (Campana, 2001) and a sharp drop in this marginal increment, once a year, is taken as an indication of annual ring formation. The analysis is often performed on pooled age classes (Barger, 1985; Manickchand-Heileman and Kenny, 1990; Murphy and Taylor, 1990; Ross et al., 1995; Pearson, 1996; Morales-Nin and Moranta, 1997; Van der Walt and Beckley, 1997) or on a restricted number of age classes (Sainsbury and Whitelaw, 1984; McPherson and Squire, 1992).

---

[1] Stephenson, P. C., and I. Dunk. 1996. Relating fishing mortality to fish trawl effort on the North West Slope of Western Australia. Final report of project 93/25 to the Fisheries Research and Development Corporation, 1995, 44 p. Western Australia Marine Research Laboratories, PO Box 20, North Beach, Western Australia 6092, Australia.

Analysis with pooled data has limited value because there may be different patterns of growth-ring formation at different life stages (e.g. at sexual maturity) and pooled data may have interage differences masked by dominant age groups (Beamish and McFarlane, 1983; Hyndes et al., 1992). Studies in which data were pooled only for young and old fish, due to low fish numbers, reduced these problems and improved the credibility of the results (Hyndes et al., 1992; Fletcher and Blight, 1996; Hesp et al., 2002).

Accounts of statistical analysis of the marginal increment data are rare. Davis and West (1992) used ANOVA to show that there were differences in the marginal increment of urohyal bones of *L. vitta* with time of year. As this seasonal pattern was the same for age classes 1 to 6, Davis and West (1992) pooled the data and used a graphical representation to show the time of formation of the annual rings.

This article describes a method for modeling changes in the index of completion of an otolith growth increment over time. This method enables quantitative determination of the most probable time of growth-ring formation (with confidence intervals) and is illustrated for the species *L. vitta*, *L.* sp. 3, *N. furcosus*, and *L. sebae*, from the Pilbara fish trawl fishery.

Materials and methods

Between October and November 1993 and between October and November 1994, samples of 30 fish of each species were randomly selected each month from fishery-independent trawl surveys. For the other months between January 1994 and March 1995, samples of 30 fish of each species were randomly selected each month from commercial catches. The samples came from an area between 115°30′E longitude and 120°E longitude; between the 50 meter and 100 meter depth isobaths.

The sagittal otoliths were extracted from each sampled fish and the right otolith was embedded in epoxy resin and then sectioned transversely through the otolith core to a thickness of 0.4 mm. A Gemmaster high speed saw with a 100 mm by 0.1 mm diamond tipped saw blade was used for sectioning. The otolith sections were set on 76 mm by 50 mm glass slides with casting resin and covered with cover slips. The sections were viewed with a dissecting microscope with an attached color video camera connected to a personal computer and a color monitor. Transmitted light revealed alternating wide opaque and narrow translucent zones. The translucent zones, referred to in the present study as growth rings, were counted to determine fish ages.

The distance from the outer extremity of the last wide, dark band to the otolith edge, w_i, is referred to as the marginal increment and the distance between the outer edges of the second to last and the last dark band is denoted by w_{i-1}. The distances w_i and w_{i-1} on the portion of the otolith ventral to the sulcus towards the proximal margin were measured on the computer screen. The index of completion, c_i, was determined by using the formula of Tanaka et al. (1981)

$$c_i = \frac{w_i}{w_i - 1} \qquad (1)$$

and written to a file by using a computer program written in the programming language "HiSoft Basic" (version 2.0. MichTron, Auburn Hills, MI).

The index of completion, c_i, we expect to increase over time, and then decrease abruptly when a new growth ring is formed. The timing of formation of a new ring would occur at the same time for a fish species with the same number of rings, but there would be considerable variability in timing and detection between species and individuals (Fig. 1).

The increase in the index of completion over time, t, is modeled as a strictly increasing function $f(t, a, b, d)$ with the following parameters: maximum value, a, rate of increase, b, and horizontal translation, d.

For our study, data were collected over a period of 18 months (October 1993 to March 1995) and the relation between the index of completion and time was expressed as two functions, denoted F_1 and F_2

$$F_1 : \hat{c}_1 = f(t, a, b, d_1) \text{ and } F_2 : \hat{c}_2 = f(t, a, b, d_2), \qquad (2)$$

where $\hat{c}_1$ and $\hat{c}_2$ = the estimates of the index of completion;
t = the time in months from $t = 0$ (1 October 1993) to $t = 18$ (31 March 1995); and
d_1, d_2 = the translation parameters for functions F_1 and F_2 respectively.

If the point (c_i, t) is associated with function F_1, the value of the normal probability density function of the observed deviation from F_1, evaluated at observation, i, is given by

$$\lambda_{1,i} = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(\frac{(c_i - \hat{c}_1)^2}{2\sigma^2}\right),$$

and similarly the value of the normal probability density function of the observed deviation from F_2, evaluated at observation, i, is given by

$$\lambda_{2,i} = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(\frac{(c_i - \hat{c}_2)^2}{2\sigma^2}\right),$$

where σ^2 is the variance of the residuals when F_1 is fitted to the data and where it is assumed to be equal to the variance of the residuals when the function F_2 is fitted.

To ensure the tractability of the subsequent analysis, we assume that the probability, P_t, of a point with index of completion c_i, at time t, being represented by F_1, is given by the logistic function

$$P_t = \frac{1}{1 + \exp\left[\ln(19)\frac{(R - t)}{(S - R)}\right]}, \qquad (3)$$

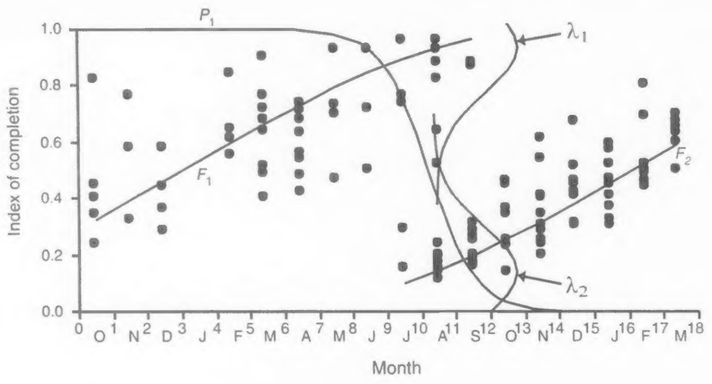


Figure 1

Values of index of completion at time t, (c_i, t) for *L. vitta* with five growth rings during the monthly time intervals from 1 October 1993 (t=0) to 31 March 1995 (t=18). Also illustrated are the functions F_1 and F_2 fitted to (C_i, t) and the function P_t representing the probability that (C_i, t) is most likely represented by function F_1. The likelihood functions, λ_1 and λ_2 are shown for the values of index of completion when t = 10.4.

where R, S, and R–(S–R) are the values of t corresponding to the 50th , 95th, and 5th percentiles of the logistic function. The probability that the index of completion is associated with F_2, rather than F_1, is calculated as $1–P_t$.

Figure 1 illustrates typical values of the index of completion at time t and the functions F_1 and F_2 representing these points before and after new growth-ring detection. The most likely time at which a new growth ring is detected is given by the value of t when P_t=0.5. The likelihood functions λ_1 and λ_2 are illustrated for t = 10.4 months. When $\lambda_{1,i}$ is high, (c_i, t) is likely to lie closest to F_1 and when $\lambda_{2,i}$ is high, (c_i, t) is likely to lie closest to F_2.

As a point (c_i, t) will be associated with either F_1 or F_2 (but not both), it follows that the likelihood function K is given by

the probability of the observed deviation from F_1, given that the point is associated with F_1	×	probability that the point is represented by F_1	+	the probability of the observed deviation from F_2, given that the point is associated with F_2.	×	probability that the point is represented by F_2.

That is, $$K_{t,i} = \lambda_{1,i} P_t + \lambda_{2,i}(1 - P_t).$$

The overall log-likelihood associated with all the observed points (c_i, t), , for i = 1 to n in a particular age class is given by

$$\sum_{i=1}^{n} \ln(K_{t,i}) = \sum_{i=1}^{n} \ln\left[\lambda_{1,i} P_t + \lambda_{2,i}(1 - P_t)\right].$$

The parameters of the functions F_1 and F_2 (i.e. a, b, d_1, and d_2, as well as σ, R, and S) were estimated separately for each value of the number of rings by maximizing the log-likelihood.

The value of $t = R$ corresponds to the month where a value of the index of completion is equally likely to be on either F_1 or F_2; that is, the point where the drop in the index of completion occurs. The value of S and R–(S–R) correspond to the 95th and the 5th percentiles for the time at which a new growth ring is likely to be detected, indicating reliability of the estimate of the time of ring formation $t = R$.

Results

The plots of index of completion versus time reflected this growth pattern in the four species we studied, with the growth rate decreasing as the time of new growth-ring detection approached.

The temporal pattern of growth of the otolith suggests the index of completion could be modeled with a logistic function

$$F(t,a,b,d) = \frac{a}{1 + \exp\left[\ln 19 \frac{(d-t)}{b}\right]},$$

with the maximum value a = 1, phase shift d, and rate of increase b.

A characteristic of otolith growth is that the distance between growth rings decreases each year, thus, the rate of increase in the marginal increments will be greater for fish with few rings and less for fish with many rings. On the other hand, the index of completion, being the ratio of

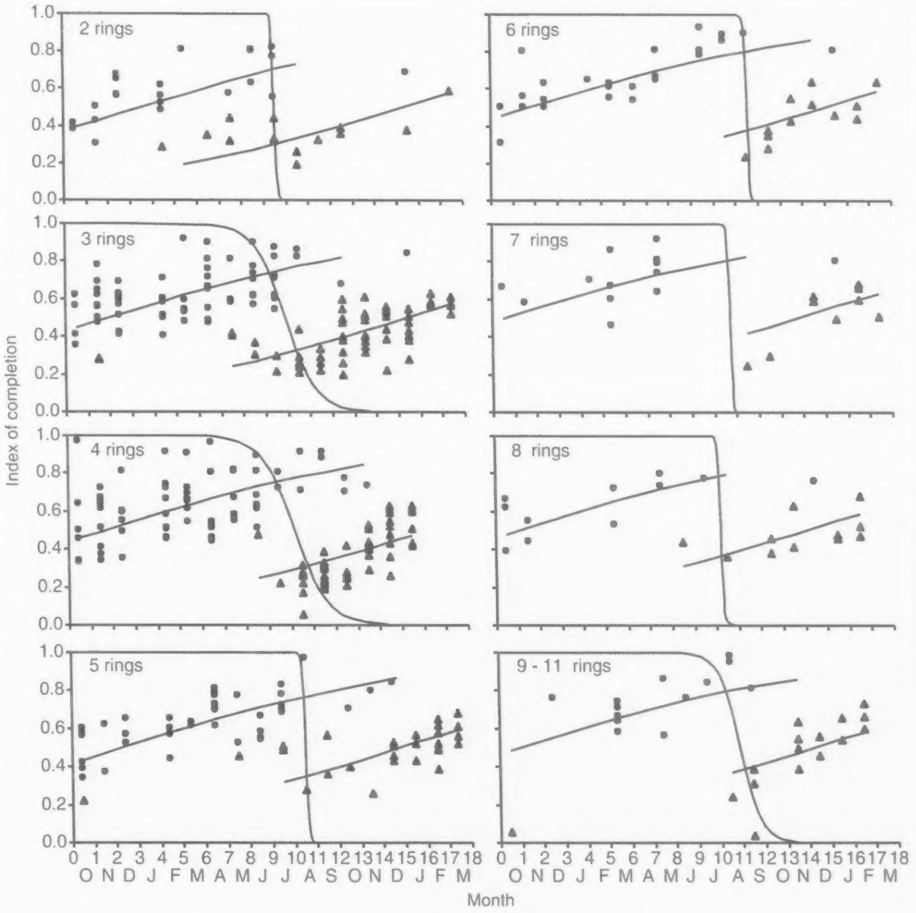


Figure 2

Index of completion at time t, (c_i, t), for *L.* sp. 3 for growth-ring categories 2, 3, 4, 5, 6, 7, 8, and 9–11 sampled between the months 1 October 1993 (t=0) to 31 March 1995 (t=18). The solid circles (●) represent those (c_i, t) most likely represented by $\hat{c}_1$ and lying closest to the function F_1 and the solid triangles (▲) are those most likely represented by $\hat{c}_2$ and lying closest to the function F_2. The logistic function, P_t, indicates the probability that points (c_i, t) are most likely represented by function F_1.

the marginal increment to the width of the previous band, would be expected to be constant at the same time of year for a particular species, regardless of the ring count.

Thus, during the maximization of the objective function

$$\sum_{i=1}^{n} \ln(K_{t,i}),$$

the rate of increase parameter b is assumed constant for all ring counts for a species, but the other parameters (d_1, d_2, R, and S) are estimated for each number of rings for each species.

Figure 2 shows the pattern of changes in the index of completion for *L.* sp. 3 for otoliths with two to eleven growth rings from 1 October 1993 (t=0) to 31 March 1995 (t=18). The data were pooled for the older age classes (nine or more growth rings) because of the small numbers of old fish in the samples. The points indicating the values of index of completion, c_i, are represented with different symbols according to whether they are most likely to be represented by $\hat{c}_1$ (lying closest to the function F_1) or most likely to be represented by $\hat{c}_2$ (lying closest to the lower line F_2). The function P_t represents the probability of points being represented by line F_1. The time where P_t=0.5 is the most likely time of detection of the formation of a new growth ring.

In the other three species, *L. vitta*, *N. furcosus*, and *L. sebae*, the points representing index of completion, (c_i, t) and functions, F_1, F_2, and P_t are illustrated in Figures 3, 4,

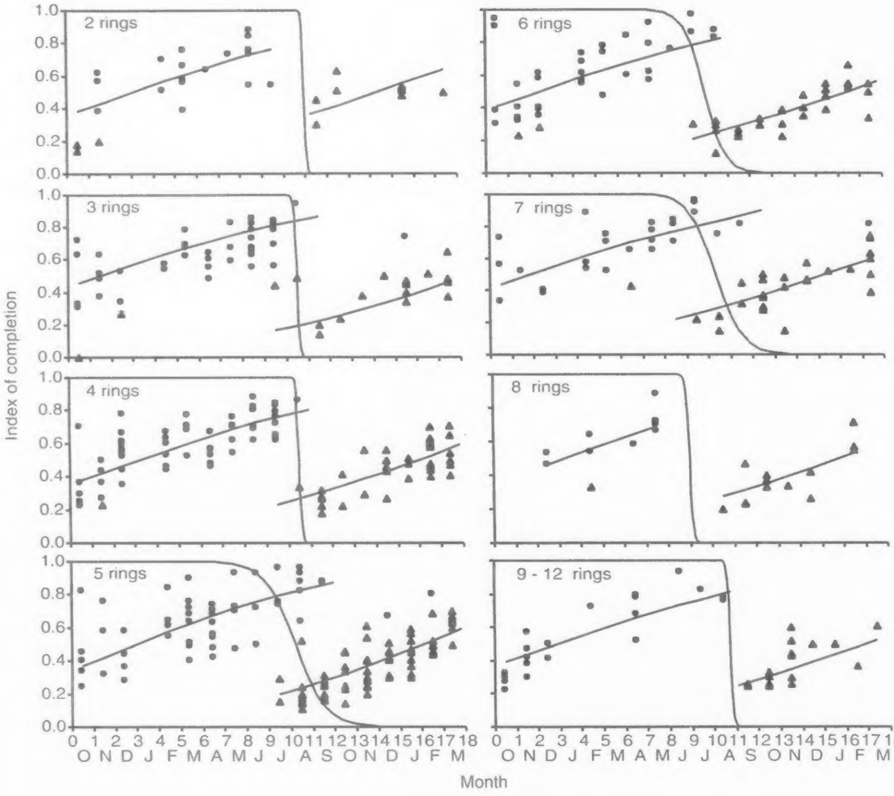


Figure 3

Index of completion at time t, (c_i, t), for *L. vitta* for growth-ring categories 2, 3, 4, 5, 6, 7, 8, and 9–12 sampled between the months 1 October 1993 ($t=0$) to 31 March 1995 ($t=18$). The solid circles (●) represent those (c_i, t) most likely represented by $\hat{c}_1$ and lying closest to the function F_1 and the solid triangles (▲) are those most likely represented by $\hat{c}_2$ and lying closest to the function F_2. The logistic function, P_t, indicates the probability that points (c_i, t) are most likely represented by function F_1.

and 5. Because of the smaller numbers of fish, the data was pooled for *L. vitta* with 9–12 growth rings, for *N. furcosus* with 7–9 growth rings, and for *L. sebae* with 13–14 and 15–19 growth rings.

The estimates of the parameters b, d_1, d_2 (the rate of increase and phase shift of F_1 and F_2), R, the time of formation of a new growth ring, with the range $\pm(S\text{–}R)$, and the standard deviation (σ) for the four species are listed in Table 1.

The estimated time of formation of a new ring varied between age classes and occurred for *L.* sp. 3 from mid July to mid September, for *L. vitta* from early July to early September, for *N. furcosus* from mid July to late September, and for *L. sebae* from mid July to mid November.

The confidence intervals are small for *L.* sp. 3 and *L. vitta* and generally larger for *N. furcosus* and *L. sebae* where reading increments was more difficult. The confidence interval was smaller for *L. sebae* with 12–19 growth rings because the clarity of the rings generally improved for older fish.

The difference between the phase shift for functions F_1 and F_2, that is d_2–d_1, was between 11 to 13 months. This difference indicated an annual cycle of growth-ring formation. The standard deviation, σ, of the values of index of completion was lowest for for *L. vitta* (0.1–0.14) and highest for *L. sebae* (0.11–0.21) and *L.* sp. 3 (0.11–0.24).

Discussion

With different starting values for the parameters, the method we described found that the time of detection of growth-ring formation for the four species was consistent. Although the timing varied considerably for different numbers of growth rings, the estimate was generally similar for each species.

Other marginal increment studies on these species produced estimates of the times for growth-ring formation

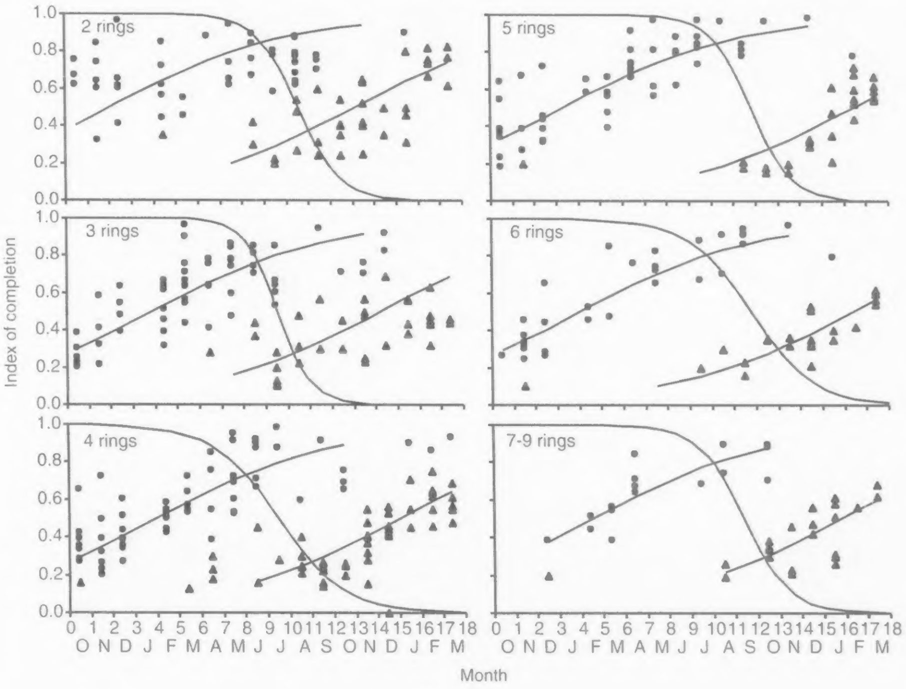


Figure 4

Index of completion at time t, (c_i, t), for *N. furcosus* for growth-ring categories 2 ,3, 4, 5, 6, and 7–9 sampled between the months 1 October 1993 (t=0) to 31 March 1995 (t=18). The solid circles (●) represent those (c_i, t), most likely represented by $\hat{c}_1$ and lying closest to the function F_1 and the solid triangles (▲) are those most likely represented by $\hat{c}_2$ and lying closest to the function F_2. The logistic function, P_t, indicates the probability that (c_i, t) are most likely represented by function F_1.

that are consistent with those determined in the present study (Table 2). Davis and West (1992) found that the time of formation of a new translucent growth-ring on urohyal bones for *L. vitta* was October, later than the timing found in our study. Sainsbury and Whitelaw (1984) found the marginal increment values on whole otoliths from *N. furcosus* had low values in July 1979 and in May 1980 (earlier than observed in the present study for sectioned otoliths) but the sampling reported by Sainsbury and Whitelaw (1984) was very sparse: four sampling times in 1979 and two in 1980. McPherson and Squire (1992) reported that the mean monthly marginal increment of the first two age classes for *L. sebae* appeared to have a minimum between July and September that is consistent with the present study.

In our study, the growth zones on *L. vitta* were generally clearly defined; the opaque zone was easily distinguished from the translucent zone, and there were few discontinuities (areas of dissimilar structure or optical density within the growth zone). This clear definition was especially noticeable for the outer growth zones of the otolith for older fish which often had very clear dark zones. This finding is consistent with the small confidence intervals for this species, especially in the fish with a greater number of growth rings in their otoliths. The growth rings on *L.* sp. 3 and *N. furcosus* had poor contrast and had many discontinuities which made the analysis difficult. For young *L. sebae*, there were many discontinuities within the growth zones which made locating the translucent zone difficult. For fish with two to four growth rings, low values of the index of completion occurred when t = 7 and also when t = 12 (Fig. 5). For the older *L. sebae* (where the number of rings is greater than or equal to 12) the wide zone was very dark and by increasing the magnification, the marginal increment could be measured relatively easily. The narrow confidence limits for the timing of growth-ring formation for *L. sebae* are consistent with this explanation but the small number of data points results in less reliable measures in the timing of new ring formation.

The time of formation of the new growth ring was slightly earlier for *L. vitta* than for *L.* sp.3, *N. furcosus,* or *L. sebae*. The calculation of an earlier growth-ring formation may be attributed to the more clearly defined translucent zone in *L. vitta* which may be detectable earlier in

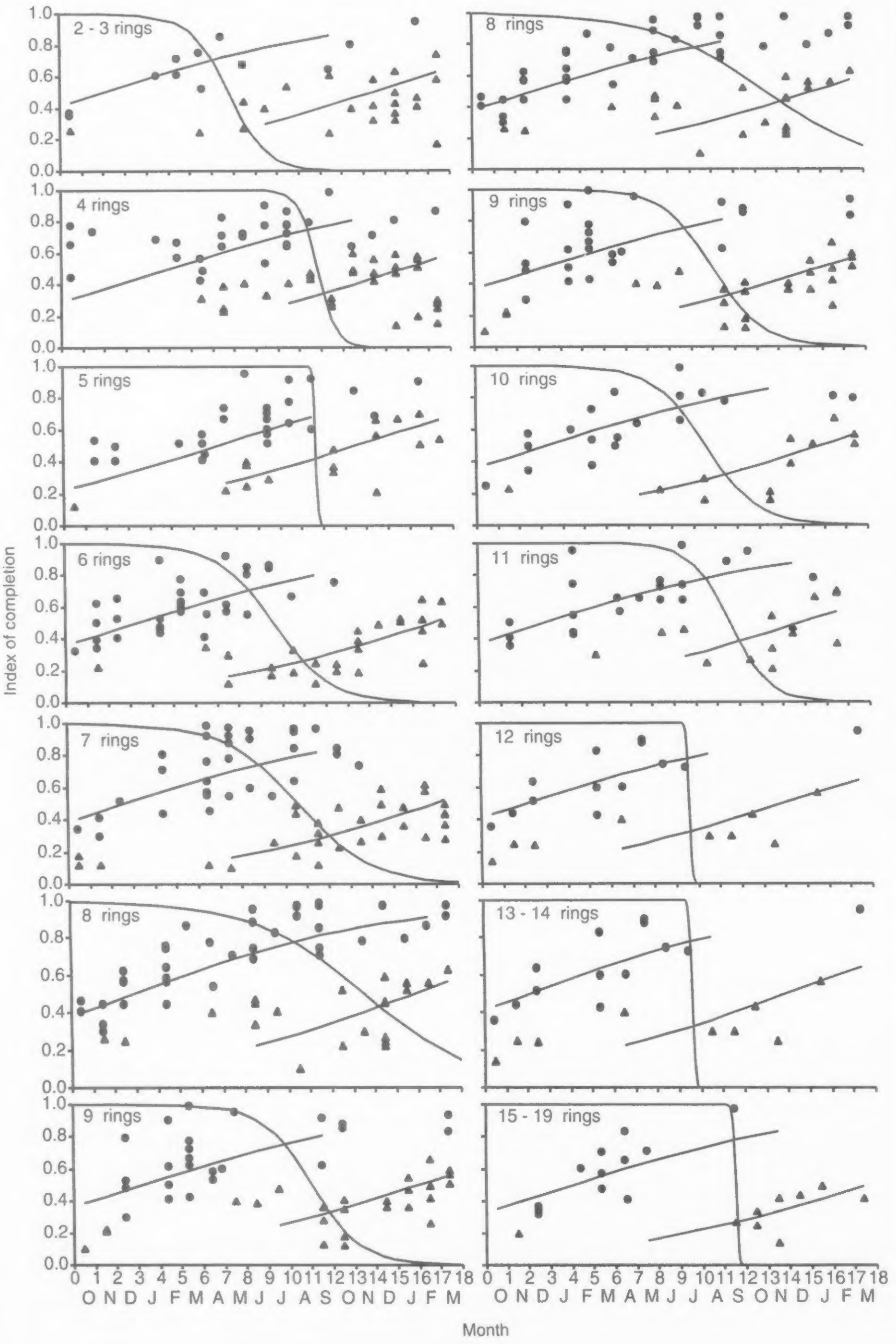


Figure 5

Index of completion at time t, (c_i, t), for *L. sebae* for growth-ring categories 2–3, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13–14, and 15–19 sampled between the months 1 October 1993 (t=0) to 31 March 1995 (t=18). The solid circles (●) represent those (c_i, t) most likely represented by $\hat{c}_1$ and lying closest to the function F_1 and the solid triangles (▲) are those most likely represented by $\hat{c}_2$ and lying closest to the function F_2. The logistic function, P_t, indicates the probability that (c_i, t) are most likely represented by function F_1.

Table 1

Parameter estimates for the fit of two functions to the index of completion data. The parameters are the rate of increase, b, the phase shifts d_1 and d_2, the time of new growth-ring detection $t = R$ with the confidence interval (5th and 95th percentiles), and the standard deviation, σ, of the observed values of index of completion for points fitted to function F_1 and F_2.

	rings	n	b	d_1	d_2	R	Month	σ
Lutjanus sp. 3	2	36	20.3	3.6	15.3	9.5 ±0.1	Jul	0.14
	3	134	20.3	2.0	15.4	9.9 ±2.0	Jul	0.13
	4	146	20.3	1.7	16.2	10.2 ±2.1	Aug	0.13
	5	72	20.3	2.3	14.8	10.5 ±0.1	Aug	0.24
	6	38	20.3	1.7	14.9	11.5 ±0.2	Sep	0.11
	7	22	20.3	0.6	13.7	10.7 ±0.1	Aug	0.12
	8	22	20.3	1.1	14.0	10.2 ±0.1	Aug	0.12
	9–11	29	20.3	0.9	14.2	10.9 ±1.3	Aug	0.15
Lutjanus vitta	2	31	15.8	3.0	14.3	10.9 ±0.1	Aug	0.13
	3	62	15.8	1.5	18.3	10.5 ±0.2	Aug	0.14
	4	96	15.8	3.4	15.9	10.5 ±0.2	Aug	0.10
	5	127	15.8	2.7	16.3	10.2 ±2.0	Aug	0.12
	6	66	15.8	2.5	16.5	10.0 ±1.3	Aug	0.12
	7	56	15.8	2.0	15.4	10.3 ±1.7	Aug	0.11
	8	24	15.8	3.3	15.8	9.0 ±0.2	Jul	0.10
	9–12	39	15.8	3.1	16.2	11.1 ±0.2	Sep	0.10
Nemipterus furcosus	2	85	11.9	2.0	13.1	10.6 ±2.7	Aug	0.18
	3	95	11.9	3.9	14.2	9.5 ±2.2	Jul	0.17
	4	116	11.9	4.0	15.0	9.5 ±4.3	Jul	0.14
	5	82	11.9	3.3	16.4	11.8 ±2.6	Sep	0.11
	6	53	11.9	4.0	16.4	11.8 ±4.2	Sep	0.10
	7–9	39	11.9	4.5	15.8	11.4 ±3.0	Sep	0.10
Lutjanus sebae	2–3	34	17.4	1.9	14.4	7.8 ±2.5	May	0.17
	4	62	17.4	5.0	15.9	11.9 ±1.2	Sep	0.21
	5	43	17.4	5.1	13.4	11.6 ±6.7	Sep	0.14
	6	60	17.4	3.3	17.0	9.6 ±3.7	Jul	0.13
	7	63	17.4	2.6	17.0	10.6 ±4.9	Aug	0.16
	8	60	17.4	2.8	15.8	13.5 ±7.6	Nov	0.15
	9	52	17.4	3.2	15.9	11.0 ±3.3	Sep	0.19
	10	32	17.4	3.3	15.9	10.6 ±3.4	Aug	0.15
	11	34	17.4	3.1	14.9	11.6 ±2.6	Sep	0.17
	12	22	17.4	2.1	13.9	9.6 ±0.1	Jul	0.12
	13–14	23	17.4	4.7	14.6	13.3 ±0.4	Nov	0.21
	15–19	21	17.4	4.0	17.6	11.5 ±0.2	Sep	0.11

Table 2

The timing of growth-ring formation for the four species in the present study and for comparative studies

	Lutjanus sp.3	*L. vitta*	*Nemipterus furcosus*	*L. sebae*
Present study	July–September	July–September	July–September	July–November
Davis and West (1992)		October		
Sainsbury and Whitelaw (1984)			May–July	
McPherson and Squire (1992)				July–September

the year than it is in the other three species. Similarly, the apparent earlier timing of new ring creation in our study, compared to the findings of Davis and West (1992) may have been due to the fact that the translucent zone can probably be detected closer to the time of formation in sectioned otoliths than in urohyals (Reshetnikov and Claro, 1976).

In summary, modeling the change in index of completion over time enabled estimates to be made of the time of the formation of a new growth ring (with confidence intervals) for four tropical species. Although the index of completion was modeled with a logistic function in the present study, alternative functions (e.g. sine or linear), gave very similar results. The technique is a useful addition to marginal increment analysis because it can be used in place of previous subjective methods to determine quantitatively the timing of new ring formation.

Acknowledgments

This project was financed by the Fisheries Research and Development Corporation (FRDC) (project 94/25) and the Department of Fisheries, Western Australia where the main author was employed for the duration of the project. The authors thank Mike Moran (Department of Fisheries, Western Australia) for obtaining FRDC funding and providing critical advice and encouragement. The senior author also thanks Robert Black (University of Western Australia) for his valued suggestions and encouragement. Stephen Newman (Department of Fisheries, Western Australia) provided advice on reading and interpretation of otolith bands, Iain Dunk (Department of Fisheries, Western Australia) collected samples, sectioned otoliths, and acted as the second reader for fish aging and marginal increment analysis. Tony Paust, Ken Bryers, Justin Chidlow, Daryn Payne (Department of Fisheries, Western Australia) assisted in sample collection. I also acknowledge the assistance of M. G. Kailis, Kraus Fishing Company, and Westmore Seafoods whose vessels were used for sample collection, and two anonymous referees for their constructive comments.

Literature cited

Beamish, R. J., and G. A. McFarlane.
1983. The forgotten requirement for age validation in fisheries biology. Trans. Am. Fish. Soc. 112:735–743.

Barger, L. E.
1985. Age and growth of Atlantic Croakers in the Northern Gulf of Mexico based on sectioned otoliths. Trans. Am. Fish. Soc. 114:847–850.

Campana, S. E.
2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish Biol. 59:197–242.

Carpenter, K. E., and V. H. Niem.
2001. FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific, vol. 5. Bony fishes, part 3 (Menidae to Pomacentridae), p. 2791–3380. FAO (Food and Agriculture Organization of the United Nations), Rome, Italy.

Davis, T. L. O., and G. J. West.
1992. Growth and mortality of *Lutjanus vittus* (Quoy and Giamard) from the North West Shelf of Australia. Fish. Bull. 90:395–404.

Ferreira, B. P., and G. R. Russ.
1992. Age, growth and mortality of the inshore coral trout *Plectropomus maculatus* (Pisces: Serranidae) from the Central Great Barrier Reef, Australia. Aust. J. Mar. Freshw. Res. 43:1301–1312.

Fletcher, W. J., and S. J. Blight.
1996. Validity of using translucent zones of otoliths to age the pilchard *Sardinops sagax neopilchardus* from Albany, Western Australia. Mar. Freshw. Res. 47:617–624.

Francis, R. I. C. C., L. J. Paul, and K. P. Mulligan.
1992. Ageing of adult snapper (*Pagrus Auratus*), from otolith annual ring counts: validation by tagging and oxytetacycline injection. Aust. J. Mar. Freshw. Res. 43:1069–1089.

Hesp, S. A., I. A. Potter, and N. G. Hall.
2002. Age and size composition, growth rate, reproductive biology, and habitats of the West Australian dhufish, *Glaucosoma hebraicum*, and their relevance to management of this species. Fish. Bull. 100:214–227.

Hyndes, G. A., N. R. Loneragan, and I. C. Potter.
1992. Influence of sectioned otoliths on marginal increment trends and age and growth estimates for the flathead *Platycephalus speculator.* Fish. Bull. 90:276–284.

Manickchand-Heileman, S. C., and J. S. Kenny.
1990. Reproduction, age, and growth of the whitemouth croaker *Micropogonias furnieri* (Desmarest 1823) in Trinidad waters. Fish. Bull. 88:523–529.

McPherson, G. R., and L. Squire.
1992. Age and growth of three dominant Lutjanus species of the Great Barrier Reef inter-reef fishery. Asian Fish. Sci. 5:25–36.

Milton, D. A., S. A. Short, M. F. O'Neill, and S. J. Blaber.
1995. Ageing of three species of tropical snapper (Lutjanidae) from the Gulf of Carpentaria, Australia, using radiometry and otolith counts. Fish. Bull. 93:103–115.

Morales-Nin, B., and J. Moranta.
1997. Life history and fishery of the common dentex (*Dentex dentex*) in Mallorca (Balearic Islands, western Mediterranean). Fish. Res. 30:67–76.

Murphy, M. D., and R. G. Taylor.
1990. Reproduction, growth and mortality of red drum *Sciaenops ocellatus* in Florida waters. Fish. Bull. 88: 531–542.

Newman, S. J., D. M. Williams, and G. R. Russ.
1996. Age validation, growth and mortality rates of the tropical snappers (Pisces: Lutjanidae) *Lutjanus adetii* (Castelnau, 1973) and *L. quinquelineatus* (Bloch, 1790) from the Central Great Barrier Reef. Aust. J. Mar. Freshw. Res. 47:575–584.

Pearson, D. E.
1996. Timing of hyline-zone formation as related to sex, location, and year of capture in otoliths of the widow rockfish, *Sebastes entomelas*. Fish. Bull. 94:190–197.

Reshetnikov, Y. S., and R. M. Claro.
1976. Cycles of biological processes in tropical fishes with reference to *Lutjanus synagris*. J. Ichthyol. 16:711–733.

Ross, L. J., T. M. Stevens, and D. S. Vaughan.
1995. Age, growth, mortality, and reproductive biology of red drums in North Carolina waters. Trans. Am. Fish. Soc. 124:37–54.

Sainsbury, K. J., and A. W. Whitelaw.
1984. Biology of Peron's threadfin bream *Nemipterus peronii* (Valenciennes), from the North West Shelf of Australia. Aust. J. Mar. Freshw. Res. 35:167–185.

Tanaka, K., Y. Mugiya, and J. Yamada.
1981. Effects of photoperiod and feeding on daily growth patterns in otoliths of juvenile *Tilapia nilotica*. Fish. Bull. 79:459–465.

Van der Walt, B.A., and L. E. Beckley.
1997. Age and growth of *Sarpa salpa* (Pisces: Sparidae) off the coast of South Africa. Fish. Res. 31:241–248.

Application of DNA-based techniques for the identification of whaler sharks (*Carcharhinus* spp.) caught in protective beach meshing and by recreational fisheries off the coast of New South Wales

Ricky W. K. Chan
School of Biological, Earth and Environmental Sciences
The University of New South Wales, UNSW
Sydney, New South Wales 2052, Australia
Present address: Educational Testing Centre
The University of New South Wales
ULD 3 East Parcel Centre
Rosebery, New South Wales 2018, Australia
E-mail address: sharkman@etc.unsw.edu.au

Patricia I. Dixon
Centre for Marine and Coastal Studies
The University of New South Wales, UNSW
Sydney, New South Wales 2052, Australia

Julian G. Pepperell
Pepperell Research and Consulting
PO Box 1475
Noosaville D.C., Queensland 4566, Australia

Dennis D. Reid
New South Wales Fisheries
PO Box 21
Cronulla, New South Wales 2230, Australia

The International Union for the Conservation of Nature's (IUCN) development of the Shark Specialist Group is indicative of the increasing environmental awareness of sharks' crucial ecological role as apex predators and that they are being threatened by human activities. Although the conservation status of certain carcharhinid species (*Carcharhinus limbatus, C. obscurus,* and *C. plumbeus*) are presently considered at low risk or near threatened according to the IUCN's threatened species categories,[1] species from the genus *Carcharhinus* are known to inhabit the waters of New South Wales (NSW), Australia (Stevens, 1984; Last and Stevens, 1994); however their conservation status has not been determined. Known as whaler or "requiem" sharks, they are also commonly caught off the coast of New South Wales in commercial fisheries (Stevens and Wayte[2]; Tanner and Liggins[3]), recreational fisheries (Pepperell, 1992; Gartside et al., 1999; Steffe et al.[4]) and by protective beach meshing (Reid and Krogh, 1992; Dudley, 1997).

Because of morphological similarities between a number of shark species in the genus *Carcharhinus* (Last and Stevens, 1994; Naylor and Marcus, 1994), taxonomic identification to species level has been difficult or inaccurate (or both)(Stevens and Wayte[2]). Historical catches of certain species of sharks in NSW commercial fisheries, recreational fisheries, and protective beach meshing have been recorded to genus level only (Pepperell, 1992; Reid and Krogh, 1992; Tanner and Liggins[3]). Formally trained Australian Fisheries Management Authority (AFMA) observers aboard commercial longlining vessels may experience difficulties in identification if distinguishing parts of a shark are discarded prior to confirmation of species (Stevens and Wayte[2]). Similarly, observers of protective beach meshing may find species identification difficult on severely decomposed sharks. Without proper identification, the exact number of individual species inhabiting NSW waters and the number of each species being landed cannot be determined (Chan, 2001).

The rise of molecular biological techniques in marine forensic science has facilitated the development of accurate taxonomic identification of shark species by sampling biological tissue (Martin, 1991; Lavery, 1992; Heist and Gold, 1999). DNA techniques require only muscle tissue, allowing biopsy tissue to be taken from specimens that can be released, rather than having to sacrifice the shark to obtain liver and heart tissue for allozyme analysis (Godfrey, 1997). Methods of taxonomic identification include PCR-based restriction fragment length poly-

---

[1] Musick, J., and S. Fowler. 2000. *Carcharhinus limbatus, C. obscurus* and *C. plumbeus* *In* IUCN 2002. 2002 IUCN red list of threatened species. http://www.iucn.org/redlist/2000index.html. [Accessed 1 October 2002.]

[2] Stevens, J. D., and S. E. Wayte. 1998. A review of Australia's pelagic sharks resources. Fisheries Research and Development Corporation project 98/107, 64 p. CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7001 Australia.

[3] Tanner, M., and G. W. Liggins. 2000. New South Wales commercial fisheries statistics 1993/94 to 1996/98, 82 p. New South Wales Fisheries, PO Box 21, Cronulla, NSW 2230 Australia.

[4] Steffe, A. S., J. J. Murphy, D. J. Chapman, B. E. Tarlinton, G. N. G. Gordon, and A. Grinberg. 1996. An assessment of the impact of offshore recreational fishing in New South Wales waters on the management of commercial fisheries. Fisheries Research and Development Corporation Project 94/053, 139 p. New South Wales Fisheries, PO Box 21, Cronulla, NSW 2230 Australia.

Manuscript approved for publication 24 June 2003 by Scientific Editor.

Manuscript received 13 July at NMFS Scientific Publications Office.

Fish. Bull. 101:910–914 (2003).

morphism (PCR-RFLP, Martin, 1991), DNA sequencing techniques (Heist and Gold, 1999), isoelectric focusing of muscle proteins (Renon et al., 2001; Smith and Benson, 2001) and direct multiplex PCR amplification (Shivji et al., 2002). These techniques use the differences in the sequences of nucleotide bases within a DNA strand among species. DNA techniques have high sensitivity, are easily reproduced, and allow the development of a unique "DNA fingerprint" for each species (Martin, 1991; Innes et al., 1998; Pepperell and Grewe, 1999). It was the aim of this project to initiate a shark DNA database for species identification of pelagic sharks (beginning with species from the genus *Carcharhinus*) in New South Wales by using PCR-RFLP techniques.

Materials and methods

Sharks of the genus *Carcharhinus* landed by recreational fisheries and caught in NSW beach meshing were identified to species level by using morphometric taxonomic guides and dentition identification (Cliff and Wilson, 1994; Last and Stevens, 1994; Naylor and Marcus, 1994). Positively identified sharks were retained as voucher specimens (see "Acknowledgments" section). A tissue biopsy (5–10 g) from the dorsal region on either side of all voucher specimens and unidentified sharks was taken and stored in 75% ethanol prior to DNA extraction. Mitochondrial DNA (mtDNA) of specimens from six species of the genus *Carcharhinus* (*C. brachyurus*, *C. brevipinna*, *C. falciformis*, *C. leucas*, *C. limbatus*, and *C. obscurus*; see Table 1 for sample sizes) was extracted by using a Fastprep DNA Extraction kit (BIO101, Integrated Sciences, Sydney, New South Wales) following the manufacturer's instructions. Approximately 200–300 mg of tissue was placed in a sterilized 1.5-mL eppendorf tube and after the addition of 1 mL Fastprep lysis buffer, incubated at 56°C for three hours prior to the extraction stage (Chan, 2001). Following the extraction procedure of the Fastprep protocol, quality and quantity of DNA was measured by using a GeneQuant DNA/RNA calculator (Amersham Biosciences, Sydney, New South Wales).

The polymerase chain reaction (PCR) was used to amplify the 1146 nucleotide base-pair (bp) cytochrome *b* (cyt *b*) region of the mtDNA (Martin and Palumbi, 1993; Kitamura et al., 1996). For each 50 µL PCR reaction, 100–200 ng of template mtDNA was used, 1.5 mM $MgCl_2$, 1X PCR buffer, 2 mM of dNTP, 5 mM of each external primer (5′–TGACTTGAARAACCAYCGTTG–3′ and 3′–CTCCAGTCTTCGRCTTACAAG–5′) and two units of DyNAzyme EXT DNA polymerase (Finnzymes, GeneWorks, Adelaide, South Australia) were added to a sterilized 200 µL PCR tube. The PCR was undertaken in a MJR MiniCycler (MJR, GeneWorks) with a heated bonnet on a cycle of 94°C for three minutes, followed by 35 cycles of 94°C denaturing for 45 seconds, 48°C annealing for 30 seconds, 72°C extension for 90 seconds, and a final 10-minute extension time at 72°C (Chan, 2001).

To determine if the cyt *b* region was successfully amplified, 10 µL of PCR product was added to 2 µL loading dye (25% bromophenol blue, 40% sucrose in distilled water) and loaded into wells of a 1.5% w/v agarose gel submerged in 0.5X TBE (Tris-borate-EDTA, pH 8) buffer, with 1 µg of a 100-bp DNA ladder (Sigma-Aldrich, Sydney, New South Wales) added to 5 µL distilled water added to each side-end well. The gel was subject to electrophoresis at 125 V for 45–60 minutes and then stained in ethidium bromide for 10 minutes, de-stained in fresh distilled water for 20 minutes prior to illumination under ultraviolet (UV) light to determine the success and yield of the amplification.

To obtain species-specific profiles, restriction fragment length polymorphism (RFLP) was used on the entire 1146 bp cyt *b* fragment (Martin, 1991; Chan, 2001). The successful amplified reaction products had the primers, taq polymerase, and buffer chemicals removed by using a BRESAspin PCR purification kit (GeneWorks). For each RFLP reaction, 30 µL of purified PCR-amplified cyt *b* mtDNA (300–1000 ng; 30 µL of distilled water was used for control reactions), 5 µL of 10X buffer, one unit of a restriction enzyme (*Alu* I, *Hae* III, *Pst* I, *Taq* I, and *Xho* I) and distilled water up to 50 µL volume was added to a sterilized 200 µL PCR reaction tube and incubated at 37°C for 2 hours in the MJR minicycler (with heated bonnet), with the exception of *Taq* I (incubated at 65°C for two hours in a water bath). After the allotted digestion time, 10 µL of loading dye was added to each tube prior to loading into a 1.5% w/v agarose gel submerged in 0.5X TBE buffer. In both end wells, 5 µL of distilled water + 1 µg of a 100 bp DNA ladder was added. The gel was subject to electrophoresis at 125 V for 60–90 minutes, stained in ethidium bromide for 10 minutes, and destained in fresh distilled water for 20 minutes prior to illumination under UV light. Enzyme-digested DNA fragments >100 bp were then "scored" to the nearest 25 bp based upon migration of the DNA fragment (the smaller the fragment, the faster the migration) and recorded for each enzyme and sample (Martin, 1991) by using the 100-bp DNA ladder as a standard measuring guide for size estimation.

Results and discussion

PCR-RFLP profiles were successfully developed for six species of the genus *Carcharhinus*; distinct and discrete patterns were observed for each species with five restriction enzymes (Table 1, and Chan, 2001). The only intraspecific polymorphism observed was for two specimens of *C. brevipinna* with the *Xho* I restriction enzyme. Increasing the sample size of all species may identify more intraspecific polymorphisms. Because of the relatively small sample sizes, no statistical analyses were undertaken. Other restriction enzymes were tested (Chan, 2001), and with the possible inclusion of other species from the genus *Carcharhinus* into this database in the future, these restriction enzymes may be required in order to discern the additional species. Because some of the fragment sizes were rounded to the nearest 25 bp, the sum of the fragments for a restriction enzyme of a species may be more than 1146 bp, the size of the cyt *b* uncleaved region for sharks (Martin and Palumbi, 1993). Fragments <100 bp were not recorded because the DNA ladder had a lower limit of 100 bp.

Table 1

Summary of PCR-RFLP banding patterns for the cytochrome *b* (cyt *b*) region in *Carcharhinus* spp. Fragment sizes are given in number of base pairs (bp) and have been rounded to the nearest 25 bp. Where the enzyme appeared not to have cleaved the cyt *b* region, it was scored "1146." *n* = denotes sample size. ✓ = denotes fragment size present.

		Carcharhinus species						
Enzyme	Fragment size	*C. limbatus* (*n*=9)	*C. brachyurus* (*n*=12)	*C. leucas* (*n*=3)	*C. obscurus* (*n*=29)	*C. falciformis* (*n*=12)	*C. brevipinna* Haplotype 1 (*n*=6)	*C. brevipinna* Haplotype 2 (*n*=2)
Alu I	1000				✓			
	700		✓					
	600						✓	✓
	500					✓		
	450	✓						
	350	✓		✓			✓	✓
	300		✓			✓		
	200				✓			
Hae III	1100	✓		✓				
	975		✓			✓	✓	✓
	750				✓			
	225				✓			
	175		✓		✓	✓	✓	✓
Pst I	1146		✓		✓	✓	✓	✓
	975	✓		✓				
	175	✓						
Taq I	1146	✓					✓	✓
	1100			✓	✓			
	850		✓					
	650					✓		
	325		✓					
	300					✓		
Xho I	1146	✓		✓	✓		✓	
	850		✓			✓		✓
	325		✓			✓		

These techniques can be used to complement morphometric identification (Cliff and Wilson, 1994; Last and Stevens, 1994; Naylor and Marcus, 1994) or can be used to identify "cryptic" species when morphological identification cannot be done. Other "cryptic" species caught in beach meshing and by recreational fisheries can be added to the DNA database, such as hammerhead sharks (*Sphyrna* spp.) which are commonly caught and are recorded in catch records to genus level only (Pepperell, 1992; Reid and Krogh, 1992; Chan, 2001). Although this project positively identified six species from the genus *Carcharhinus*, other species of this genus are known to inhabit the NSW coastline (Stevens, 1984; Last and Stevens, 1994). During the warmer months, when the northern currents extend farther south to the Sydney region, transient tropical *Carcharhinus* spp. may appear off the coast. In the northern regions of NSW, there have been recorded catches of the blacktip reef shark (*C. melanopterus*) by shore-based anglers (Gartside et al., 1999). Although transient tropical whaler sharks may not have permanent stocks in NSW waters, it is important to discern them from resident *Carcharhinus* spp. prior to any species-specific stock assessment. Given the number of shark species and difference in life histories (Last and Stevens, 1994; Smith et al., 1998), identification to species level is crucial.

The use of genetic techniques allows, for the first time, accurate identification of species of whaler sharks that were landed by recreational fisheries and caught in protective beach meshing in NSW and that have been historically recorded to genus level. Continual sampling and formal identification are required for comparison of catches between species of *Carcharhinus*. Genetic techniques have the potential to be used for all other shark species and fisheries within the Australian Fishing Zone (AFZ). The use of genetic techniques has been employed in the field of law enforcement to prevent the selling of protected fish species at local fish markets where the majority of the carcass is not retained (Ward et al., 1999). This use could be extended to ensure that protected shark species such as the grey nurse shark (*Carcharias taurus*), white shark (*Carcharodon carcharias*), and the smalltooth sand tiger shark (*Odontaspis ferox*) are not sold.

This project is the first time that *Carcharhinus* spp. have been formally identified to species level in the 60-year history of NSW protective beach meshing and only the second time in NSW recreational fisheries after Stevens (1984). The depositing of voucher specimens and all DNA biopsies at the Australian Museum ensures that these valuable and irreplaceable biological samples can be used in future research. It is evident that DNA techniques can be used to taxonomically identify "cryptic" specimens, especially *Carcharhinus* spp., and *Sphyrna* spp. to species level that were once recorded to genus level only in many fisheries based in NSW (Pepperell, 1992; Reid and Krogh, 1992; Chan, 2001; Tanner and Liggins[3]). It is important that sharks that are caught be recorded to the lowest taxonomic level for management and conservation strategies. Long-term routine sampling and recording to species level will provide useful data on which conservation management strategies can be developed as part of the Australian national plan of action for the conservation and management of sharks.

Acknowledgments

The authors would like to acknowledge the assistance of the NSW Game Fishing Association and all NSW recreational gamefishing clubs, their officials and their anglers who cooperated with the research, NSW protective beach meshing contractors and observers, NSW Fisheries staff, and the numerous volunteers (Joanne Bennett, Tanya Compton, Rikkè Dano, Paul Godfrey, Gary Henry, Andrew Hodges, Alex Irwin, Jeff Murphy, Julie Needham, Milena Rantala, and Clint Wilson) who helped collect samples. We thank Ed Heist and Andrew Martin for their comments on the manuscript and specially thank Bill Sherwin (UNSW) and Marie Roseline Yardin for their assistance in this project. This project was funded by NSW Fisheries and the National Heritage Trust Coast and Clean Seas' Marine Species Protection Program (CCS Project no. 9856). Voucher shark specimens were retained at the Australian Museum, Sydney, NSW, Australia (Collection Manager, Fish Section) and NSW Fisheries, NSW, Australia (Dennis Reid).

Literature cited

Chan, R.W. K.
2001. Biological studies on sharks caught off New South Wales. Ph.D. diss., 314 p. School of Biological Science, Univ. New South Wales, Sydney, Australia.

Cliff, G., and R. B. Wilson.
1994. Natal Sharks Board's field guide to sharks and other marine animals, 57 p. Group Editors, Durban, South Africa.

Dudley, S. F. J.
1997. A comparison of the shark control programs of New South Wales and Queensland (Australia) and KwaZulu-Natal (South Africa). Ocean Coast. Manag. 34:1–27.

Gartside, D. F., B. Harrison, and B. L. Ryan.
1999. An evaluation of the use of fishing club records in the management of marine recreational fisheries. Fish. Res. 41:47–61.

Godfrey, P.
1997. Identification of sharks caught in NSW waters using allozyme electrophoresis. B.S. (Hons.) thesis, 54 p. Centre for Marine and Coastal Studies, Univ. New South Wales, Sydney, Australia.

Heist, E. J., and J. R. Gold.
1999. Genetic identification of sharks in the U.S. Atlantic large coastal shark fishery. Fish. Bull. 97:53–61.

Innes, B. H., P. M. Grewe, and R. D. Ward.
1998. PCR-based genetic identification of marlin and other billfish. Mar. Freshw. Res. 49:383–388.

Kitamura, T., A. Takemura, S. Watabe, T. Taniuchi, and M. Shimizu.
1996. Mitochondrial DNA analysis for the cytochrome *b* gene and D-loop region from the bull shark *Carcharhinus leucas*. Fish. Sci. 62:22–27.

Last, P. R., and J. D. Stevens.
1994. Sharks and rays of Australia, 513 p. CSIRO (Commonwealth Scientific and Industrial Research Organisation) Publishing, Melbourne, Australia.

Lavery, S.
1992. Electrophoretic analysis of phylogenetic relationships among Australian carcharhinid sharks. *In* Sharks: biology and fisheries (J. G. Pepperell, ed.), p. 97–108. Aust. J. Mar. Freshw. Res. 43.

Martin, A. P.
1991. Application of mitochondrial DNA sequence analysis to the problem of species identification of sharks. *In* Conservation biology of elasmobranchs (S. Branstetter, ed.), p. 53–59. NOAA Tech. Rep. NMFS 115.

Martin, A. P., and S. R. Palumbi.
1993. Protein evolution in different cellular environments: cytochrome *b* in sharks and mammals. Mol. Biol. Evol. 10: 873–891.

Naylor, G. J. P., and L. F. Marcus.
1994. Identifying isolated shark teeth of the genus *Carcharhinus* to species: relevance for tracking phyletic change through the fossil record. Am. Mus. Novit. 94:1–53.

Pepperell, J. G.
1992. Trends in the distribution, species composition and size of sharks caught by gamefish anglers off South-eastern Australia, 1961–90. *In* Sharks: biology and fisheries (J. G. Pepperell, ed.), p. 213–225. Aust. J. Mar. Freshw. Res. 43.

Pepperell, J. G., and P. M. Grewe.
1999. A field guide to Indo-Pacific billfishes, 16 p. CSIRO Publishing, Melbourne, Australia.

Reid, D. D., and M. Krogh.
1992. Assessment of catches from protective shark meshing off New South Wales beaches between 1950 and 1990. *In* Sharks: biology and fisheries (J. G. Pepperell, ed.), p. 283–296. Aust. J. Mar. Freshw. Res. 43.

Renon, P., M. M. Colombo, F. Colombo, R. Malandra, and P. A. Biondi.
2001. Computer-assisted evaluation of isoelectric focusing patterns in electrophoretic gels: identification of smoothhounds (*Mustelus mustelus*, *Mustelus asterias*) and comparison with lower value shark species. Electrophoresis 22:1534–1538.

Shivji, M., S. Clarke, M. Pank, L. Natanson, N. Kohler, and M. Stanhope.
2002. Genetic identification of pelagic shark body parts for

conservation and trade monitoring. Conserv. Biol. 16: 1036–1047.

Smith, P. J., and P. G. Benson.
2001. Biochemical identification of shark fins and fillets from the coastal fisheries in New Zealand. Fish. Bull. 99: 351–355.

Smith, S. E., D. W. Au, and C. Show.
1998. Intrinsic rebound potentials of 26 species of Pacific sharks. Mar. Freshw. Res. 49:663–678.

Stevens, J. D.
1984. Biological observations on sharks caught by sport fishermen off New South Wales. Aust. J. Mar. Freshw. Res. 35:573–590.

Ward, R. D., R. K. Daley, J. Andrew, and G. K. Yearsley.
1999. Protein fingerprinting. *In* Australian seafood handbook: an identification guide to domestic species (G. K. Yearsley, P. R. Last, and R. D. Ward, eds.), chap. 9, p. 358–392. CSIRO Marine Research, Hobart, Australia.

Red sea urchins (*Strongylocentrotus franciscanus*) can live over 100 years: confirmation with A-bomb ^{14}carbon

Thomas A. Ebert
Department of Zoology
Oregon State University
Corvallis, Oregon 97331-2914
E-mail address: ebertt@sciences.oregonstate.edu

John R. Southon
Center for Accelerator Mass Spectrometry
Lawrence Livermore National Laboratory
Livermore, California 94551-9900

Red sea urchins (*Strongylocentrotus franciscanus*) along the west coast of North America, like most large sea urchins in temperate waters worldwide, are the focus of a commercially important fishery. In a review of biological data for purposes of fishery management, the life span of red sea urchins was suggested to be 7–10 years (Sloan, 1986) and they have been included with much shorter-lived species for illustrating complex population dynamics (Hastings and Higgins, 1994). Recent work with tetracycline and calcein tagging (Ebert, 1998; Ebert et al., 1999), however, has shown that individuals continue to grow throughout life, although at a very slow rate, and large individuals are estimated to be in excess of 100 years old. A potential problem with the studies using tetracycline and calcein is that one-year time intervals were used between tagging and recapture and therefore it is possible that occasionally there may have been very good years for growth that were missed. If occasional growth spurts occurred, largest sizes would have been attained in much less than 100 years. The potential problem of missed good years for growth could be resolved with a marker that captures a longer period of time. The accuracy of age estimates has consequences for resource management where size limits may need adjustment in order to protect older individuals (Hilborn and Walters, 1992; Congdon et al., 1994; Ebert, 1998). There is also the need to understand the evolution of life histories of species where long life tends to be an indicator of uncertainty in individual reproductive success (Murphy, 1968; Roff, 1992; Stearns, 1992).

Enhanced radiocarbon in the oceans due to atmospheric testing of nuclear weapons that began in the 1950s (Nydal and Lovseth, 1983; Broecker et al., 1985, Duffy et al., 1995) provides a permanent marker in carbonate-based skeletal elements that are not reworked by resorption and deposition during growth and hence has a long time period between mark and recovery. The enhanced radiocarbon marker has been used in various studies to validate the periodic (usually annual) nature of growth zones in fish (Kalish, 1993, 1995; Campana, 1997; Campana et al., 2002) and invertebrates (Turekian et al., 1982; Witbaard et al., 1994; Peck and Brey, 1996) where validation by chemical tags such as tetracycline has been impractical. Red sea urchins lack interpretable growth zones (Breen and Adkins, 1976) and therefore there is no natural feature to serve as a cross check for studies using chemical tags. In the present study we present a test and confirmation of age in red sea urchins estimated from tetracycline tagging using an enhanced ^{14}C signal in the ocean from nuclear weapons testing.

Materials and methods

Red sea urchins were tagged with tetracycline from 1989 to 1992 in northern California, Oregon, and Washington and collected after time intervals of approximately one year (details presented in Ebert et al., 1999). It is not possible to determine whether a live sea urchin has a tetracycline mark and therefore large collections had to be made. Skeletal elements were cleaned with sodium hypochlorite bleach to remove all organic material not bound in the calcite of the skeleton, and then skeletal ossicles were examined by using UV illumination to detect the tetracycline marks, which fluoresce yellow. Growth increments were measured in jaws of Aristotle's lantern of 1582 tagged-recovered red sea urchins and used to estimate growth parameters. Jaw ossicles, the demipyramids of Aristotle's lantern, are internal skeletal elements that grow around all surfaces but not equally in all directions so that a change in jaw length, ΔJ, is mostly at the end closest to the esophagus and there is little growth closest to the mouth, the labial end, where the teeth extend from the jaw.

The Tanaka function (Eq. 1) was used to describe growth (Tanaka, 1982, 1988) because it can model data that show an initial lag, an exponential phase with a maximum, and can include continuing growth throughout life. This function is described in greater detail elsewhere (Tanaka, 1982, 1988; Ebert et al., 1999). The usual formulation of the Tanaka model is Δsize as a function of size at time t and Δt is assumed to be fixed for all individuals in the sample, usually at $\Delta t = 1$ year (Tanaka, 1982, 1988; Ebert, 1998; Ebert et al., 1999) and not included explicitly in the equation. In the present study we estimated the amount of jaw that would have to be removed to represent the time span from the time of collection in the 1990s with relatively high ^{14}C levels to the time before atmospheric testing of atomic bombs (relatively low ^{14}C) and

Manuscript approved for publication 10 July 2003 by Scientific Editor.
Manuscript received 13 July 2003 at NMFS Scientific Publications Office.
Fish. Bull. 101(4):915–922 (2003).

therefore the Tanaka model was modified from previous uses to make ΔJ a function of $J_{t+\Delta t}$, the size on the date of recapture rather the date of marking, which is the usual way of estimating growth parameters. Also, Δt was explicitly included as a variable (Eq. 2),

$$\Delta J = J_{t+\Delta_t} - \frac{1}{\sqrt{f}} \ln\left[2G + 2\sqrt{G^2 + fa}\right] - d \tag{1}$$

where

$$G = E/4 - fa/E - f\Delta t \tag{2}$$

and

$$E = \exp\left(\sqrt{f}\,(J_{t+\Delta t} - d)\right). \tag{3}$$

The three parameters of the Tanaka function, *a*, *d*, and *f*, have biological meaning: "*a*" is related to maximum growth rate, which is approximately $1\sqrt{a}$; "*d*" shifts the size at which growth is maximum; and "*f*" is a measure of the rate of change of the growth rate. A graphical presentation of how changes in these parameters change the growth curve is given in Ebert et al. (1999).

Explicit use of Δt and making ΔJ a function of $J_{t+\Delta t}$ required a modification of the usual presentation of the Tanaka function. In Ebert et al. (1999) Equation 2 was written as

$$G = E/4 - fa/E + f \tag{4}$$

with no Δt and with "+ *f*". Equation 3 was written as

$$E = \exp\left(\sqrt{f}\,(J_t - d)\right). \tag{5}$$

with J_t, rather than $J_{t+\Delta t}$. Tetracycline tagging for a period of one year, $\Delta t = 1$, provides the Tanaka parameter estimates and these parameters were used to estimate a Δjaw size that would cover the time from the date of collection to a time, Δt, before A-bomb testing; Δt is time run backwards from the date of collection, which is the reason for the sign change from Equation 4 to Equation 2.

The samples of red sea urchins that were selected for radiocarbon analysis were part of the tagging study at Halftide Rocks off San Juan Island, Washington (Ebert et al., 1999). Individuals were tagged with tetracycline on 26 October 1991 and collected again on 21 October 1992. The recaptured tagged individuals (n=365) are part of the 1582 tagged sea urchins from northern California, Oregon, and Washington that were used to estimate Tanaka parameters. For ^{14}C analysis, specimens were selected from the Halftide Rocks collection that did not show fluorescence in the skeleton and therefore probably had not been handled in 1991. The use of untagged individuals for radiocarbon analyses avoids any possible contamination from handling and tagging in 1991.

Cleaned jaws for ^{14}C analysis were cemented to aluminum blocks with a two-part epoxy cement and aligned so that the esophageal margin was approximately parallel with the block base. The block was held on the stage of a small milling machine and the stage tilted so that the jaw was as parallel as possible with the milling bit. Approximately 0.5 mm of the jaw surface was removed and sides were milled to remove recently deposited calcite and to expose the underlying older skeleton. The jaw was measured and successive samples were milled from the esophageal edge to a depth of 0.5 mm, which produced samples larger than 1 mg of carbonate in most cases. Samples were placed in individual reaction chambers, evacuated, acidified with orthophosphoric acid, and heated. The evolved CO_2 was converted to graphite by reduction with an excess of hydrogen in individual reactors with iron powder as a catalyst (Vogel et al., 1987). Analysis of ^{14}C in the graphite targets was done at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, and reported as Δ^{14}C‰ (Stuiver and Polach, 1977), which includes a correction for a δ^{13}C of −3 based on stable isotope analyses. Mean precision (1 standard deviation) of radiocarbon measurements was 4.2‰ (range: 3.0–7.9).

Results

Of the 1582 tag recoveries from all sites, 739 jaws showed a growth increment, ΔJ, of $\leq$0.02 cm and of these only 13 had a labial measurement >0, which is at the end of the jaw at the mouth opening. The smallest nonzero measurements were 0.001 cm and therefore growth less that this was recorded as 0; 54 sea urchins in the sample had clear tetracycline marks but 0 measurable growth. For large jaws, the measured labial component was too small to be measured and therefore all of the calculated ΔJ since the late 1950s was milled from the esophageal end of the jaw only.

Tetracycline tagging indicated that annual jaw growth (Fig. 1) was very slow for large sea urchins and many individuals showed annual increments of less than 0.01 cm. The resulting growth curve of jaw length as a function of age (Fig. 2A) showed that at least some large individuals would be expected to have ages in excess of 100 years. If this age estimate is correct, a drop in ^{14}C should be found in successive small slices removed from large jaws, which would first show current ^{14}C levels and then drop to prebomb levels. Because the Halftide Rocks samples were collected in 1992 we used Δt = 35 years, which would go back to 1957. Using Equations 1–3, growth parameters given in Fig. 1, and Δt = 35 years, we estimated the increment to be between 1 and 2 mm for jaws between 2.5 and 2.6 cm (Fig. 2B).

Successive milled samples from the esophageal ends of large jaws (Fig. 3, A–D) showed a precipitous drop in radiocarbon to prebomb levels over 1–2 millimeters, in agreement with predictions. Variations across replicates and samples probably are the result of differences in the width of milled samples and an inability to remove all recently deposited calcite or to follow the exact growing edge of the jaw with the milling machine. Smaller jaws (Fig. 3, E–G) were not expected to show a prebomb signature, and indeed they did not. They do, however, indicate the ^{14}C level to be expected in recent skeletal material and emphasize the rapid change in radiocarbon shown in large jaws. Changes in ^{14}C in successive milled samples in jaws

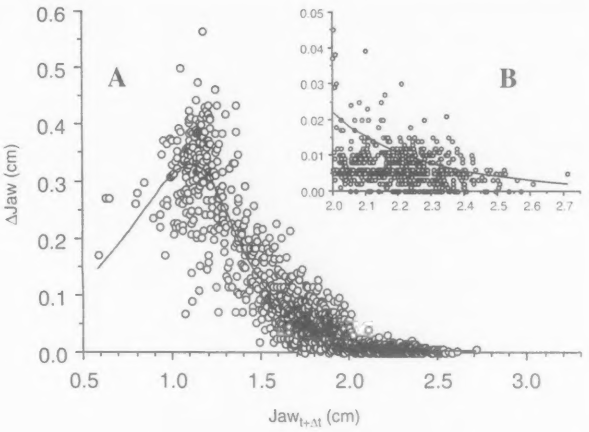


Figure 1

Jaw growth increments, ΔJ, for tagged red sea urchins (*Strongylocentrotus franciscanus*) from northern California, Oregon, and Washington; fitted line is the Tanaka function (Tanaka, 1982, 1988; Ebert et al., 1999) with $f = 10.95650 \pm 0.35064$ SE(standard error), $d = 0.04937 \pm 0.01664$, and $a = 8.63029 \pm 0.16659$; Δt is approximately 1 year for all samples; (**A**) entire data set; $n = 1582$; (**B**) restricted scale to show just jaws larger than 2.0 cm and degree of scatter; $n = 336$; conversion to body diameter, D, from jaw length, J, is $D = 4.8951J^{1.2817}$; therefore jaw lengths of 2.0 and 2.7 cm would have expected test diameters of 11.9 and 17.9 cm, respectively.

E–G are similar to changes shown in coral samples from the Galápagos (Guilderson and Schrag, 1998) and may indicate that ^{14}C levels in surface waters in regions of strong upwelling were still rising when sea urchin were collected in 1992. The conclusion is that ^{14}C analysis supports the age estimates based on tetracycline tagging and use of the Tanaka function: large red sea urchins are old and may have ages of 100 years or more

Discussion

The largest reported red sea urchins, with body diameters over 19 cm, are from British Columbia, Canada, (Bureau, 1996) and with estimated jaw lengths of about 2.8 cm would be expected to be around 200 years old (Fig. 2A). Age estimates of 100+ years far exceed estimates of life span for other sea urchins (Table 1) based on growth lines in ossicles. Natural growth lines, however, tend to underestimate ages of old individuals because very small increments will have alternations of dark and light areas that are difficult or impossible to resolve and hence counts underestimate age (Ebert, 1988). For example, the maximum age estimate for *Strongylocentrotus droebachiensis*, the commercial species of the U.S. east coast, is 25 years by counts of growth lines (Robinson and MacIntyre, 1997) but at least twice this if tagging and size structure (Russell et al., 1998) are used. Similarly, tagging and size structure of *Evechinus chloroticus* (Lamare and Mladenov, 2000) have indicated survival rates similar to *S. franciscanus* but the maximum number of growth lines reported was only 10 (Dix, 1972). Survival rate, however, is not a fixed parameter for a species and there is local variation, as well as geographic patterns, evident in the survival rate for *S. franciscanus* (Ebert et al. 1999).

Estimates of annual survival rates based on growth parameters and mean size for red sea urchins from southern California to Alaska (Ebert et al., 1999) indicate that very old individuals would not be expected in southern California where few individuals attain ages of 50 years. At more northern locations, the probability of long life increases (Fig. 4) and ages of 100+ are expected, particularly in Washington and Alaska. The mechanism causing the latitudinal pattern are unclear. Latitudinal differences in survival may be due to increased disease outbreaks associated with higher temperatures in the south (Ebert et al. 1999) or the presence of more predator species in the south (Tegner, 2001). Physiological senescence related to temperature is unlikely because there is no pattern to growth differences associated with latitude (Ebert et al., 1999) and no evidence for physiological decline in relative gonad size in the south (Tegner and Levin, 1983) or north (Kramer and Nordin, 1975). The largest individuals continue to develop gonad masses in accord with the same allometric relationships as smaller individuals. It is reasonable to conclude that senescence does not occur in red sea urchins.

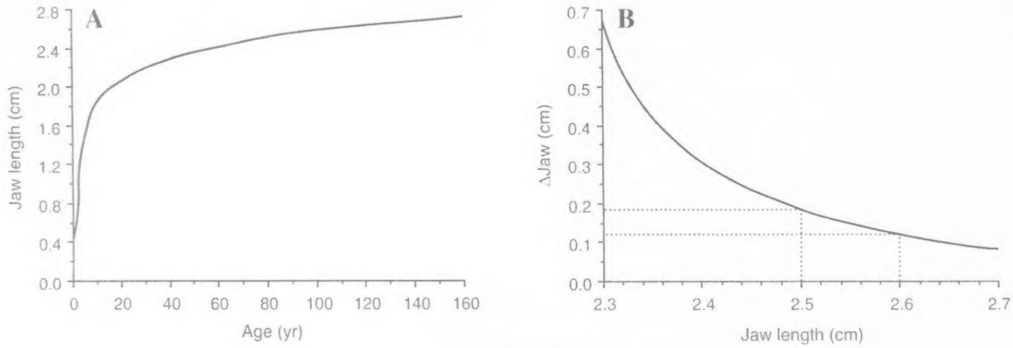


Figure 2

(**A**) Growth curve for jaws of red sea urchins from northern California, Oregon, and Washington using Tanaka parameters and an initial jaw size of 0.44 cm, the approximate jaw size for a 1-year-old red sea urchin; (**B**) change in jaw size, *Δjaw*, with *Δtime* = 35 years starting with a final jaw length; *Δjaw* is the estimate of how much of the jaw would have to be removed to expose prebomb calcite for sea urchins collected in 1992.

Table 1

Maximum age estimates for sea urchins based on growth zones in skeletal ossicles.

Species	Years	Reference
Lytechinus variegatus	4	Beddingfield and McClintock (2000)
Strongylocentrotus nudus	6	Kawamura (1966)
Psammechinus miliaris	7	Jensen (1969)
Paracentrotus lividus	8	Crapp and Willis (1975)
Sphaerechinus granularis	9	Lumingas and Guillou (1994)
Evechinus chloroticus	10	Dix (1972)
Psammechinus miliaris	10	Gage (1991)
Strongylocentrotus intermedius	10	Agatsuma (2001)
Echinus acutus var. *norvegicus*	11	Gage et al. (1986)
Loxechinus albus	11	Gebauer and Moreno (1995)
Echinus esculentus	12	Nichols et al. (1985)
Allocentrotus fragilis	15	Sumich and McCauley (1973)
Echinus elegans	21	Gage et al. (1986)
Strongylocentrotus droebachiensis	24	Robinson and MacIntyre (1997)
Echinus affinis	28	Gage and Tyler (1985)
Sterechinus neumayeri	40	Brey et al. (1995)
Sterechinus antarcticus	75	Brey (1991)

Red sea urchins larvae spend at least two months in the plankton (Strathmann, 1978) during which time they can be carried far along the coast or out to sea. There is year-to-year variation in settlement and recruitment and years of zero success and greater variation at northern sites (Bernard and Miller, 1973, Low, 1975, Tegner and Dayton, 1981, Duggins, 1983, Pearse and Hines, 1987, Sloan et al., 1987, Ebert et al., 1994). An important point, however, is that these authors reported some recruitment at study sites and so extreme longevity would at first seem unnecessary for species survival. The important issue for evolution of life histories, however, is not whether some individuals recruit to the population but how successful an individual is each year in leaving offspring. The long life of adult red sea urchins emphasizes the difficulties individuals have in successfully having offspring that settle in suitable habitat and survive to reproductive age. Many annual reproductive episodes appear to be required to succeed and therefore red sea urchins are classic bet hedgers that use resources to promote annual survival of adults as well as to reproduce (Stearns, 1992).

Attributes of a long life span have consequences for resource management. The implications for management of the red sea urchin resource have been explored by us-

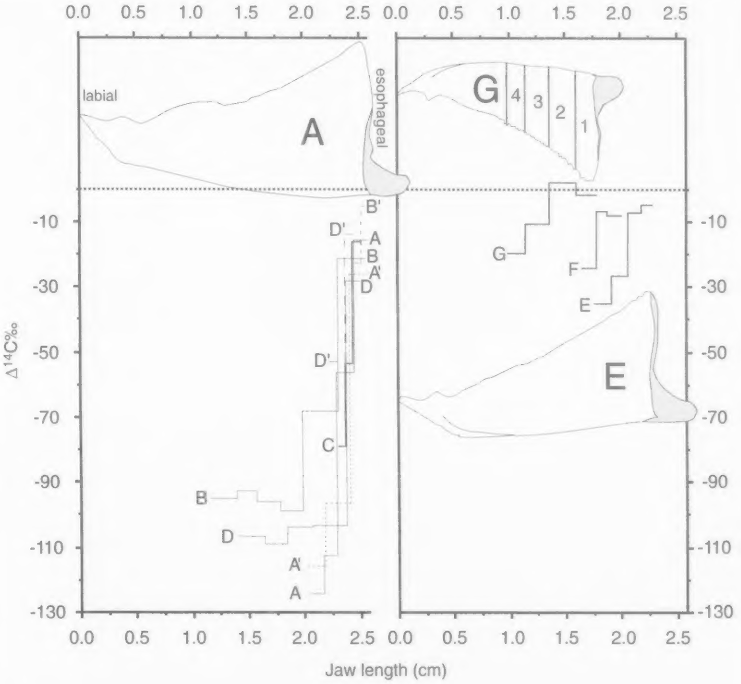


Figure 3

Change in Δ^{14}C ‰ with successive milled samples from the esophageal end of red sea urchin jaws collected at Halftide Rocks, Washington (48°28.8′N, 1220°59.8′W), 21 Oct. 1992. Step width in lines is the width of a milled sample. Jaws of three sea urchins are drawn but a total of 7 were sampled; jaw lengths are A = 2.58 cm, B = 2.54, C = 2.53, D = 2.51, E = 2.29, F = 2.01, G = 1.80 cm; primes represent replicate jaws from the same sea urchin; jaw C is labeled just at the final milled sample (initial sample is just below the initial sample for jaw A); bands labeled 1 through 4 on jaw G show how samples were milled from the jaw after the surface layer had been removed by approximately 0.5 mm. Shaded areas of the jaw, which were not sampled, indicate an area below the top surface where a shelf exists for articulation with the epiphysis, another ossicle of Aristotle's lantern.

ing elasticity analysis (de Kroon et al., 1986) of a matrix model and have shown that small changes in survival of individuals larger than 9 cm would have a greater effect on population maintenance than survival of smaller sea urchins (Ebert, 1998). The conclusion from matrix analysis, which is supported by the ^{14}C test of growth and age of *Strongylocentrotus franciscanus* we present in our study, is that the preservation of large individuals must be included in long-term management plans for this species as well as for other long-lived sea urchins in developing fisheries such as that for *Evechinus chloroticus* (Barker, 2001). Finally, our work strongly suggests that life spans of other exploited sea urchin species should be explored in greater detail in developing management plans because preservation of large and old individuals may be very important for the long-term viability of these fisheries.

Acknowledgments

Tagging and processing sea urchins were done in collaboration with S. Schroeter and J. Dixon, with support from the Pacific States Fishery Commission, a self-imposed landing tax of sea urchin fishermen administered by the Calif. Dept. Fish & Game, Oregon Sea Urchin Community Commission, and Ore. State Univ. Sea Grant; field work was facilitated by resource managers in California, Oregon, and Washington (P. Kalvass, N. Richmond, A. Bradbury). Stable isotope analysis was done at the University of California at Davis and radiocarbon analysis was funded by a Center for Accelerator Mass Spectrometry minigrant at Lawrence Livermore National Laboratory and was carried out under the auspices of the US DOE. The manuscript benefited from a critical reading by G. Fox.

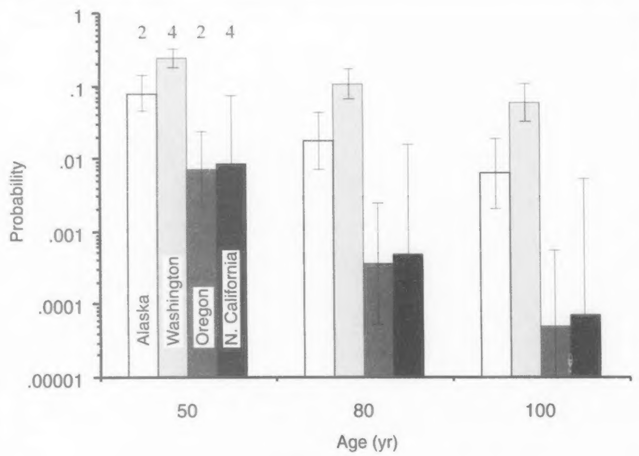


Figure 4

Probabilities of attaining ages of 50, 80, and 100 years for red sea urchins (*Strongylocentrotus franciscanus*) at sites from Alaska to northern California; estimates were based on Tanaka growth parameters and size-frequency distributions (Ebert et al., 1999); numbers of sites are shown at the tops of bars and there are two samples at each site; error bars are 2 SEs.

Literature cited

Agatsuma, Y.
2001. Ecology of *Strongylocentrotus intermedius*. *In* Edible sea urchins: biology and ecology (J. M. Lawrence, ed.), p. 333–346. Developments in aquaculture and fisheries science, no. 32. Elsevier Science B. V., Amsterdam, Netherlands.

Barker, M. F.
2001. The ecology of *Evechinus chloroticus*. *In* Edible sea urchins: biology and ecology (J. M. Lawrence, ed.), p. 245–260. Developments in aquaculture and fisheries science, no. 32. Elsevier Science B. V., Amsterdam, Netherlands.

Beddingfield, S. D., and J. B. McClintock.
2000. Demographic characteristics of *Lytechinus variegatus* (Echinoidea: Echinodermata) from three habitats in a north Florida bay, Gulf of Mexico. Mar. Ecol. 21:17–40.

Bernard, F. R., and D. C. Miller.
1973. Preliminary investigation on the red sea urchin resources of British Columbia [*Strongylocentrotus franciscanus* (Agassiz)]. Fish. Res. Board Can. Tech. Rep. 400: 1–37.

Breen, P. A., and B. E. Adkins.
1976. Growth rings and age in the red sea urchin, *Strongylocentrotus franciscanus*. Fish. Res. Board Can. Manuscr. Rep. Ser. 1413.

Brey, T.
1991. Population dynamics of *Sterechinus antarcticus* (Echinodermata: Echinoidea) on the Weddell Sea shelf and slope, Antarct. Antarctic Sci. 3:251–256.

Brey, T., J. Pearse, L. Basch, J. McClintock, and M. Slattery.
1995. Growth and production of *Sterechinus neumayeri* (Echinoidea: Echinodermata) in McMurdo Sound, Antarct. Mar. Biol. 124:279–292.

Broecker, W. S., T.-H. Peng, G. Ostlund, and M. Stuiver.
1985. The distribution of bomb radiocarbon in the ocean. J. Geophys. Res. 90:6953–6970.

Bureau, D.
1996. Relationship between feeding, reproductive condition, jaw size and density in the red sea urchin, *Strongylocentrotus franciscanus*. M.S. thesis, 90 p. Simon Fraser Univ., Burnaby, Canada.

Campana, S. E.
1997. Use of radiocarbon from nuclear fallout as a dated marker in the otoliths of haddock *Melanogrammus aeglifinus*. Mar. Ecol. Prog. Ser. 150:49–56.

Campana, S. E., L. J. Natanson, and S. Myklevoll.
2002. Bomb dating and age determination of large pelagic sharks. Can. J. Fish. Aquat. Sci. 59:450–455.

Congdon, J. D., A. E. Dunham, and R. C. van Loben Sels.
1994. Demographics of common snapping turtles (*Chelydra serpentiana*): implications for conservation and management of long-lived organisms. Am. Zool. 34:397–408.

Crapp, G. B., and M. E. Willis.
1975. Age determination in the sea urchin *Paracentrotus lividus* (Lamarck), with notes on the reproductive cycle. J. Exp. Mar. Biol. Ecol. 20:157–178.

de Kroon, H., A. Plaisier, J. van Groenendael, and H. Caswell.
1986. Elasticity: the relative contribution of demographic parameters to population growth rate. Ecology 67: 1427–1431.

Dix, T. G.
1972. Biology of *Evechinus chloroticus* (Echinoidea: Echinometridae) from different localities. 4. Age, growth, and size. N. Z. J. Mar. Freshw. Res. 6:48–68.

Duffy, P. B., D. E. Eliason, A. J. Bourgeois, and C. C. Covey.
1995. Simulation of bomb radiocarbon in two global ocean general circulation models. J. Geophys. Res. 100:22545–22563.

Duggins, D. O.
1983. Starfish predation and the creation of mosaic patterns in a kelp-dominated community. Ecology 64:1610–1619.

Ebert, T. A.
1988. Calibration of natural growth lines in ossicles of two sea urchins, *Strongylocentrotus purpuratus* and *Echinometra mathaei*, using tetracycline. *In* Echinoderm biology: proceedings of the sixth international echinoderm conference (R. D. Burke, P. V. Mladenov, P. Lambert, and R. L. Parsley, eds.), p. 435–443. A. A. Balkema, Rotterdam, Netherlands.
1998. An analysis of the importance of Allee effects in management of the red sea urchin *Strongylocentrotus franciscanus*. *In* Echinoderms: San Francisco. Proceedings, 9th international echinoderm conference (R. Mooi and M. Telford, eds.), p. 619–627. A. A. Balkema, Rotterdam, Netherlands.

Ebert, T. A., J. D. Dixon, S. C. Schroeter, P. E. Kalvass, N. T. Richmond, W. A. Bradbury, and D. A. Woodby.
1999. Growth and mortality of red sea urchins *Strongylocentrotus franciscanus* across a latitudinal gradient. Mar. Ecol. Prog. Ser. 190:189–209.

Ebert, T. A., S. C. Schroeter, J. D. Dixon, and P. E. Kalvass.
1994. Settlement patterns of red and purple sea urchins (*Strongylocentrotus franciscanus* and *S. purpuratus*) in California, USA. Mar. Ecol. Prog. Ser. 111:41–52.

Gage, J. D.
1991. Skeletal growth zones as age-markers in the sea urchin *Psammechinus miliaris*. Mar. Biol. 110:217–228.

Gage, J. D., and P. A. Tyler.
1985. Growth and recruitment of the deep-sea urchin *Echinus affinis*. Mar. Biol. 90:41–53.

Gage, J. D., P. A. Tyler, and D. Nichols.
1986. Reproduction and growth of *Echinus acutus* var. *norvegicus* Duben & Kören and *E. elegans* Duben & Kören on the continental slope off Scotland. J. Exp. Mar. Biol. Ecol. 101:61–83.

Gebauer, P., and C. A. Moreno.
1995. Experimental validation of the growth rings of *Loxechinus albus* (Molina, 1782) in southern Chile (Echinodermata: Echinoidea). Fish. Res. 21:423–435.

Guilderson, T. P., and D. P. Schrag.
1998. Abrupt shift in subsurface temperatures in the tropical Pacific associated with changes in El Niño. Science 281:240–243.

Hastings, A., and K. Higgins.
1994. Persistence of transients in spatially structured ecological models. Science 263:1133–1136.

Hilborn, R., and C. J. Walters.
1992. Quantitative fisheries stock assessment: choice, dynamics, and uncertainty, 570 p. Chapman & Hall, New York, NY.

Jensen, M.
1969. Breeding and growth of *Psammechinus miliaris* (Gmelin). Ophelia 7:65–78.

Kalish, J. M.
1993. Pre- and post-bomb radiocarbon in fish otoliths. Earth Planet. Sci. Lett. 114:549–554.
1995. Radiocarbon and fish biology. *In* Recent developments in fish otolith research (D. H. Secor, J. M. Dean, and S. E. Campana, eds.), p. 637–653. Univ. South Carolina Press, Columbia, SC.

Kawamura, K.
1966. On the age determining character and growth of a sea urchin, *Strongylocentrotus nudus*. Sci. Rep. Hokkaido Fish. Exp. Station 6:56–61.

Kramer, D. E., and D. M. A. Nordin.
1975. Physical data from a study of size, weight and gonad quality for the red sea urchin (*Strongylocentrotus franciscanus* (Agassiz)) over a one-year period. Fish. Res. Board Can. Manuscr. Rep. Ser. 1372:68.

Lamare, M. D., and P. V. Mladenov.
2000. Modelling somatic growth in the sea urchin *Evechins chloroticus* (Echinoidea: Echinometridae). J. Exp. Mar. Biol. Ecol. 243:17–43.

Low, C. J.
1975. The effect of grouping of *Strongylocentrotus franciscanus*, the giant red sea urchin, on its population biology. Ph.D. diss., 157 p. Univ. British Columbia, Vancouver, Canada.

Lumingas, L. J. L., and M. Guillou.
1994. Growth zones and back-calculation for the sea urchin, *Sphaerechinus granularis*, from the Bay of Brest, France. J. Mar. Biol. Assoc. U. K. 74:671–686.

Murphy, G. I.
1968. Pattern in life history and the environment. Am. Nat. 102:391–411.

Nichols, D., A. A. T. Sime, and G. M. Bishop.
1985. Growth in populations of the sea-urchin *Echinus esculentus* L. (Echinodermata: Echinoidea) from the English Channel and Firth of Clyde. J. Exp. Mar. Biol. Ecol. 86:219–228.

Nydal, R., and K. Lovseth.
1983. Tracing bomb ^{14}C in the atmosphere 1962–1980. J. Geophys. Res. 88:3621–3621.

Pearse, J. S., and A. H. Hines.
1987. Long-term population dynamics of sea urchins in a central California kelp forest: rare recruitment and rapid decline. Mar. Ecol. Prog. Ser. 39:275–283.

Peck, L. S., and T. Brey.
1996. Bomb signals in old Antarctic brachiopods. Nature 380:207–208.

Robinson, S. M. C., and A. D. MacIntyre.
1997. Aging and growth of the green sea urchin Bull. Aquacul. Assoc. Can. 91:56–60.

Roff, D. A.
1992. The evolution of life histories, 535 p. Chapman & Hall, New York, NY.

Russell, M. P., T. A. Ebert, and P. S. Petraitis.
1998. Field estimates of growth and mortality of the green sea urchin, *Strongylocentrotus droebachiensis*. Ophelia 48:137–153.

Sloan, N. A.
1986. Red sea urchin. Underwater world. DFO/2322 UW/ 53, 4 p. Fisheries and Oceans Canada, Ottawa, Canada.

Sloan, N. A., C. P. Lauridsen, and R. M. Harbo.
1987. Recruitment characteristics of the commercially harvested red sea urchin *Strongylocentrotus franciscanus* in southern British Columbia, Canada. Fish. Res. 5:55–69.

Stearns, S. C.
1992. The evolution of life histories, 249 p. Oxford Univ. Press, New York, NY.

Strathmann, R.
1978. Length of pelagic period in echinoderms with feeding larvae from the northwest Pacific. J. Exp. Mar. Biol. Ecol. 34:23–27.

Stuiver, M., and H. A. Polach
1977. Discussion: reporting of ^{14}C data. Radiocarbon 19: 355–363.

Sumich, J. L., and J. E. McCauley
1973. Growth of a sea urchin, *Allocentrotus fragilis*, off the Oregon Coast. Pac. Sci. 27:156–167.

Tanaka, M.

1982. A new growth curve which expresses infinite increase. Publ. Amakusa Mar. Biol. Lab. 6:167–177.

1988. Eco-physiological meaning of parameters of ALOG growth curve. Publ. Amakusa Mar. Biol. Lab. 9:103–106.

Tegner, M. J.

2001. The ecology of *Strongylocentrotus franciscanus* and *Strongylocentrotus purpuratus*. *In* Edible sea urchins: biology and ecology (J. M. Lawrence, ed.), p. 307–332. Developments in aquaculture and fisheries science, no. 32. Elsevier Science B. V., Amsterdam, Netherlands.

Tegner, M. J., and P. K. Dayton.

1981. Population structure, recruitment and mortality of two sea urchins (*Strongylocentrotus franciscanus* and *S. purpuratus*) in a kelp forest. Mar. Ecol. Prog. Ser. 5: 255–268.

Tegner, M. J., and L. A. Levin.

1983. Spiny lobsters and sea urchins: analysis of a predator-prey interaction. J. Exp. Mar. Biol. Ecol. 73:125–150.

Turekian, K. K., J. K. Cochran,. Y. Nozaki, I. Thompson, and D. S. Jones.

1982. Determination of shell deposition rates of *Arctica islandica* from the New York Bight using natural ^{228}Ra and ^{228}Th and bomb produced ^{14}C. Limnol. Oceanogr. 27: 737–741.

Vogel, J. S., J. R. Southon, and D. E. Nelson.

1987. Catalyst and binder effects in the use of filamentous graphite for AMS. Nucl. Instru. Methods Phys. Res. Sec. B 29:50–56.

Witbaard, R., M. I. Jenness, K. van der Borg, and G. Ganssen.

1994. Verification of annual growth increments in *Arctica islandica* L. from the North Sea by means of oxygen and carbon isotopes. Neth. J. Sea. Res. 33:91–101.

Abundance and distribution of cetaceans in outer continental shelf waters of the U.S. Gulf of Mexico

Gregory L. Fulling
Keith D. Mullin
Carrie W. Hubard
Southeast Fisheries Science Center
National Marine Fisheries Service, NOAA
3209 Frederic Street
Pascagoula, Mississippi 39567
E-mail address (for G. L. Fulling): Greg.Fulling@noaa.gov

The U.S. Marine Mammal Protection Act (MMPA) requires that stocks of marine mammal species in U.S. waters be maintained at or above their optimum sustainable population (OSP) level, defined as the number of animals that results in the maximum net productivity. To meet this requirement for each stock, the U.S. National Marine Fisheries Service (NMFS) estimates annual human-caused mortality and potential biological removal (PBR), the maximum number of animals that may be removed from a stock due to human activities (e.g. fisheries bycatch) while allowing the stock to reach or maintain its OSP. PBR is calculated by following specific criteria and using the estimated abundance of the stock, its maximum net productivity rate (theoretical or estimated), and a recovery factor (Barlow et al., 1995; Wade and Angliss, 1997). The NMFS is required to prepare an annual stock assessment report (SAR) for each stock to update abundance, stock structure, maximum net productivity, human-caused mortality, PBR, and status (e.g. Waring et al., 2001).

Cetaceans in the U.S. Gulf of Mexico (U.S. GOM) occur in two species assemblages that overlap in upper continental slope waters (~200–1000 m). The oceanic waters (>200 m) are routinely inhabited by 20 species that, in most cases, inhabit deep warm-temperate to tropical waters throughout the world. Bottlenose dolphins (*Tursiops truncatus*) and Atlantic spotted dolphins (*Stenella frontalis*) are the only two species commonly found in continental shelf waters (<200 m) (Mullin and Hansen, 1999).

In the U.S. GOM the distribution of *T. truncatus* ranges from inshore waters to deep waters of the continental slope (Blaylock and Hoggard, 1994; Hansen et al.[1]; Mullin and Hoggard[2]). In the U.S. GOM, the NMFS divides *T. truncatus* into 38 stocks: 33 inshore stocks (bays, sounds, and estuaries); 3 coastal stocks (western, northern, and eastern) from shore to 9 km seaward of the 18-m (10-fm) isobath; 1 outer continental shelf (OCS) stock from the coastal boundary to 9 km seaward of the 183-m (100-fm) isobath; and 1 continental shelf edge and slope stock from the OCS boundary out to the U.S. Exclusive Economic Zone (EEZ) (Waring et al., 2001). The abundance estimate for the OCS *T. truncatus* stock is 50,247 dolphins (CV=0.18) and is based on aerial surveys conducted during fall which covered all the U.S. GOM shelf waters over 3 years in sections, west, central, and east, in 1992, 1993, and 1994, respectively (Blaylock and Hoggard, 1994; Waring et al., 2001).

One U.S. GOM *S. frontalis* stock is recognized, and the abundance, 3213 dolphins (CV=0.44), is estimated from ship surveys of shelf edge and oceanic waters >100 m deep conducted from 1991–94 (Hansen et al.[1]). Abundance estimates for *S. frontalis* for the U.S. GOM OCS were not made from the 1992–94 aerial surveys although *S. frontalis* groups were sighted (Waring et al., 2001). The majority of *S. frontalis* are thought to inhabit the shelf-edge region. However, data from opportunistic sightings (e.g. Mills and Rademacher, 1996) and a summer 1994 ship survey of the eastern GOM (Hofstetter, 2002) have indicated that they are common throughout eastern GOM shelf waters >10 m deep, and in oceanic waters <500 m.

The NMFS Southeast Fisheries Science Center (SEFSC) conducts annual spring and fall ichthyoplankton surveys in the U.S. GOM. The spring survey targets the entire oceanic portion of the U.S. GOM, and the fall survey focuses on shelf waters from the U.S.-Mexico border to southern Florida. Since 1991, abundance estimates of oceanic cetacean species in the U.S. GOM have been based primarily on data collected during annual spring surveys (Hansen et al.[1]; Mullin and Hoggard[2]; Mullin and Fulling[3]). Because of the lack of current assessment information on and the uncertainty of abundance estimates for *T. truncatus* and *S. frontalis* in OCS waters, cetacean surveys were conducted during the fall ichthyoplankton surveys from 1998 to 2001. From these surveys, we report the abundance and distribution of cetaceans in OCS waters (20–200 m deep) of the U.S. GOM.

---

[1] Hansen, L. J., K. D. Mullin, and C. L. Roden. 1995. Unpublished report. Estimates of cetacean abundance in the northern Gulf of Mexico from vessel surveys, 20 p. Southeast Fisheries Science Center, 3209 Frederic St., Pascagoula, MS 39567.

[2] Mullin, K. D., and W. Hoggard. 2000. Visual surveys of cetaceans and sea turtles from aircraft and ships. *In* Cetaceans, sea turtles and seabirds in the northern Gulf of Mexico: Distribution, abundance and habitat associations. Volume II: Technical report (R.W. Davis, W. E. Evans, and D. Würsig, eds.), p. 111–172. OCS Study MMS 96-0027. Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA. 70123.

[3] Mullin, K. D., and G. L. Fulling. 2003. Unpublished report. Abundance of cetaceans in the oceanic northern Gulf of Mexico, 1996–2001, 35 p. Southeast Fisheries Science Center, 3209 Frederic Street, Pascagoula, MS 39567.

Manuscript approved for publication 10 July 2003 by Scientific Editor.
Manuscript received 20 July 2003 at NMFS Scientific Publications Office.
Fish. Bull. 101:923–932 (2003).

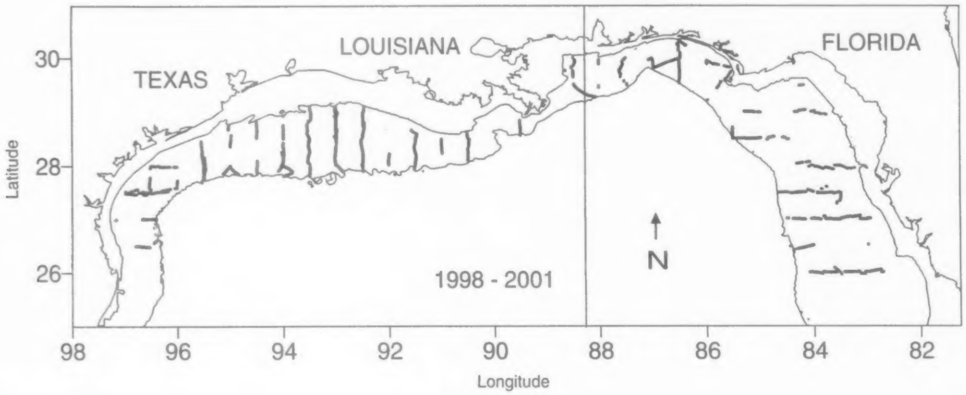


Figure 1

Survey effort in Beaufort sea state of ≤3 (dark lines), east (1342 km) and west (2202 km) of Mobile Bay, Alabama (bold vertical line), in the northern U.S. Gulf of Mexico outer continental shelf (20–200 m) during fall 1998–2001. The 20- and 200-m isobaths (thin lines) are shown.

Methods

Study area

The study area (245,800 km^2) included continental shelf waters of the U.S. GOM between the U.S-Mexico border and Key West, Florida, between the 20- and 200-m isobaths (Fig. 1). However, survey effort did not extend south of 26.0°N in the southeastern GOM and therefore abundance estimates were extrapolated for this region. The shelf is wide (up to 200 km) off the Florida peninsula and off northern Texas and Louisiana, and narrower off the Florida Panhandle near DeSoto Canyon, the Mississippi River Delta, and southern Texas. The continental slope is a steep escarpment from 1000 to 2000 m in the eastern GOM.

Survey design and data collection

Surveys were conducted from the 68-m NOAA Ship *Gordon Gunter* (1998, 1999, and 2001) and the 52-m NOAA Ship *Oregon II* (2000). The four surveys ranged from 28 to 32 days between 28 August and 2 October and were divided into two legs of 12 to 19 days. Standard ship-based, line-transect survey methods for cetaceans, similar to those used in the Pacific Ocean and U.S. GOM, were used (e.g. Barlow, 1995; Hansen et al.[4]). Surveys were conducted 24 hours a day along a predetermined trackline between plankton stations uniformly spaced 30 nmi apart. The trackline uniformly covered the shelf waters roughly 10–200 m deep in 1998–2001 (Fig. 1).

Data were collected by two teams of three observer—one team positioned on the flying bridge 14.5 m above the waterline (*Gunter*) and the other team positioned 9.2 m above the waterline (*Oregon II*) during daylight hours while the vessels moved between plankton stations, weather permitting (i.e. no rain, Beaufort sea state <6). Each team had at least two members experienced in ship-based, line-transect methods and in identification of tropical cetaceans. The left- and right-side observers searched to the horizon in the arc from 10° right and left of the ship's bow to the left and right beams (90°), respectively, using 25× binoculars. The third observer searched, using unaided eye or 7× hand-held binoculars, and recorded data. Observers changed position every 30–40 minutes, and the two teams alternated 2-h watches throughout daylight hours. Survey speed was usually 18 km/h (~10 knots) but varied with sea conditions.

Data were recorded on a computer interfaced with a global positioning system (GPS) by an in-house BASIC data acquisition program (Southeast Fisheries Science Center, NMFS, Pascagoula, MS). For each cetacean sighting, time, position, bearing and reticle (a measure of radial distance) of the sighting, species, group-size, behavior, bottom depth, sea surface temperature, and associated animals (e.g. seabirds, fish) were recorded. The bearing and radial distance for groups sighted without 25× binoculars and close to the ship were estimated. Survey effort data were automatically recorded every 2 minutes and included the ship's position and direction, effort status, observer positions, and environmental conditions that could affect the observers' ability to sight animals (e.g. Beaufort sea state, sun position). Typically, if a sighting was within a 5.5-km strip on either side of the ship, the ship was diverted from the trackline to approach the group to allow the observers to identify species and estimate group-size by consensus.

Cetaceans were identified to the lowest taxonomic level possible. Observers' ability to make identifications depended on weather and animal behavior. Differences between *T. truncatus* and *S. frontalis* could not always be distinguished at long distances and were therefore sometimes recorded as "*T. truncatus* + *S. frontalis*."

---

[4] Hansen, L. J., K. D. Mullin, T. A. Jefferson, and G. P. Scott. 1996. Visual surveys aboard ships and aircraft. *In* Distribution and abundance of marine mammals in the north-central and western Gulf of Mexico: Final report. Volume II: Technical report (R.W. Davis and G.S. Fargion, eds.), p. 55–132. OCS Study MMS 96-0027. Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA. 70123.

Analytical techniques

Survey effort that was parallel to the bathymetry gradients, occurred in waters outside the OCS study area, or occurred in a Beaufort sea state ≥4 was excluded from analyses (Fig. 1). Survey effort used in analyses is summarized in Table 1. Survey effort was not uniformly distributed throughout the study area due to poor survey conditions, particularly in the eastern GOM, during two of the four years. Because *S. frontalis* sightings were clearly more numerous in the east, we delineated the study area into west (106,186 km^2) and east (139,614 km^2) regions at 88°15.0′W (ca. Mobile Bay, Alabama) and estimated abundances separately for each region. A combination of line-transect and strip-transect methods were used to make abundance estimates. Line-transect methods were used for sightings detected with 25(binoculars, which constituted the majority of sightings (129/140). Strip-transect methods were used for the 11 sightings that were made without the 25× binoculars (naked-eye sightings) and that were observed by the primary team.

Table 1

Total survey effort (km) during 1998–2001 in waters 20–200 m and under Beaufort sea state conditions ≤3.

Year	West	East	Total
1998	174	67	241
1999	477	120	597
2000	281	0	281
2001	448	629	1077
Total	1380	816	2196

Line-transect estimates

For each species or species group (i) [i.e. *T. truncatus*, *S. frontalis*, rough-toothed dolphins (*Steno bredanensis*) and *T. truncatus*+*S. frontalis*] detected by 25× binoculars, and for each region (j) (east and west), abundance estimates were made with line-transect methods ($N_{Li,j}$) by using the software program DISTANCE (Colorado Coop. Fish and Wildlife Research Unit, Colorado State Univ., Fort Collins, CO) (Laake et al., 1993; Buckland et al., 2001) and by incorporating data into the following equation:

$$N_{Li,j} = \frac{A_j \cdot n_{Li,j} \cdot S_{Li,j} \cdot f_i(0)}{2 \cdot L_j \cdot g(0)}, \qquad (1)$$

where A_j = area of region j;
$n_{Li,j}$ = number of group sightings of species i in region j;
$S_{Li,j}$ = mean group size of species i in region j;
$f_i(0)$ = sighting probability density function at perpendicular distance zero for species i;
L_j = total length of transect line in region j; and
$g(0)$ = probability of seeing a group on the transect line.

The parameter $g(0)$ was not estimated; $g(0) = 1$ was used for each abundance estimate. Abundances were negatively biased because observers usually miss some groups at the surface on the transect line, and some groups were under the surface while in the observation area, therefore $g(0) < 1$ (see "Discussion" section). The log-normal 95% confidence interval was computed (Buckland et al., 2001) for each abundance estimate because it was a product of estimates and tended to have a skewed distribution. The variance of $N_{L\,i,\,j}$ was estimated by using

$$\operatorname{var}(N_{Li,j}) = N^2_{Li,j}\left(\frac{\operatorname{var}(n_{Li,j})}{n^2_{L_{i,j}}} + \frac{\operatorname{var}(S_{Li,j})}{S^2_{L_{i,j}}} + \frac{\operatorname{var}[f_i(0)]}{f_i(0)^2}\right). \qquad (2)$$

The sampling unit was the length of the transect completed on-effort each day with Beaufort sea state ≤3 in a region. The formula used to estimate each component of the variance is given in Buckland et al. (2001). Var($n_{L\,i,\,j}$) was length-weighted and based on the variation in the number of on-effort group sightings between sampling units that ranged up to 191 km/d.

Estimation of *f*(0)

The perpendicular distance (y) was estimated by using bearing and reticle measurements. The reticle readings were converted to radial sighting distances (R) by the method of Lerczak and Hobbs (1998; y=R sin(b), where b=angle between the sighting and the transect line). Because of the difference in observer height (5.3 m) between the *Oregon II* and *Gunter*, each ship could potentially yield a different sighting function, *g(x)*. However, only seven sightings were made in sea states ≤3 from the *Oregon II* during the one year it was used; therefore data from both ships were pooled. Estimates of $f_i(0)$ were made by using a hazard-rate, uniform, or half-normal model with exact perpendicular sighting distances and no adjustments. Model selection was determined by using Akaike's Information Criterion (AIC; Buckland et al., 2001).

The number of *S. bredanensis* groups and the number of *T. truncatus*+*S. frontalis* groups sighted was insufficient to estimate $f(0)$ for each. Because the *S. bredanensis* group and *T. truncatus*+*S. frontalis* group had similar sighting characteristics (e.g. body size, group-size, surface behavior), we pooled them with sightings of *T. truncatus* to estimate $f_i(0)$. Total number of sightings for both *T. truncatus* and *S. frontalis* was sufficient to estimate $f_i(0)$ for each without pooling with other species. Truncation for *T. truncatus*, *S. bredanensis*, and *T. truncatus* + *S. frontalis* was 3300 m, and was 5000 m for *S. frontalis*. Each estimate of $f_i(0)$ was based on pooled sightings from the east and west regions.

Estimation of mean group-size

Group-sizes tend to be related to y, because in many cases larger groups are easier to see than small groups with increasing y. In general, the arithmetic mean of group-size may be an overestimate of the true mean group-size and could lead to positively-biased abundance estimates. Therefore, a regression of group-size by y was used to estimate an "expected mean group-size" (program DISTANCE) and it was used if the regression was significant ($P<0.15$). Var(S_L) was the analytical variance for mean group-sizes based on arithmetic means or was estimated as in Buckland et al. (2001:74) for expected mean group-sizes.

Strip-transect estimates

One requirement for unbiased line-transect estimates of abundance is that the cetacean group should not move in response to the ship before it is sighted (Buckland et al., 2001). If cetaceans are not sighted before they respond to the ship, in cases of attraction to the ship, $f(0)$ and abundance will be overestimated. During previous U.S. GOM surveys, groups of *T. truncatus* or *S. frontalis* were consistently attracted to ride the bow waves as the ship approached (Würsig et al., 1998). Therefore, the abundance and variance of groups sighted by naked eye (N_S) were estimated by

$$N_{Si,j} = \frac{A_j \cdot n_{Si,j} \cdot S_{Si,j}}{2 \cdot L_j \cdot w_i} \qquad (3)$$

and

$$\mathrm{var}(N_{Si,j}) = N^2_{Si,j}\left(\frac{\mathrm{var}(n_{Si,j})}{n^2_{S_{i,j}}} + \frac{\mathrm{var}(S_{Si,j})}{S^2_{S_{i,j}}}\right), \qquad (4)$$

where $w_i = 1/f_i(0)$ which was treated as a constant, i.e. strip width, w_i, was equal to the line-transect effective strip half-width $[1/f_i(0)]$ with $\mathrm{var}(w_i) = 0$.

For each region, species total abundance ($N_{T\,i,j}$) was the line-transect and strip-transect estimates added, $N_{T\,i,j} = N_{L\,i,j} + N_{S\,i,j}$. Total U.S. GOM OCS abundance for each species was $N_{T\,i} = \Sigma N_{T\,i,j}$. The coefficient of variation (CV) for each abundance was estimated as $\mathrm{CV}(N) = [\mathrm{var}(N)]^{1/2}N$ and the CV for each summed abundance as

$$CV(N_{sum}) = \left(\sum CV^2(N) \cdot N^2\right)^{1/2} \Big/ \sum N. \qquad (5)$$

Results

Abundance estimates were based on 2196 km of effort and 140 sightings (Figs. 1 and 2). For east and west regions, there was 816 km of effort and 73 sightings, and 1380 km of effort and 67 sightings, respectively (Tables 1 and 2). Only three cetacean species were encountered. Groups of *T. truncatus* (30 east region, 45 west region) and *S. frontalis* (34 east, 12 west) were the most frequently encountered (Fig. 2, Table 2) and *S. bredanensis* groups (1 east, 2 west) were also sighted. *Tursiops truncatus* and *S. frontalis* were estimated to have $f(0)$ of 0.6238/km (CV=0.12) and 0.4101/km (CV=0.11), and an effective strip half-width of 1603 and 2438 m, respectively (Figs. 3 and 4). *Steno bredanensis* and *T. truncatus+S. frontalis* abundances were based on an $f(0)$ = 0.6059/km (CV=0.11) and an effective strip half-width of 1650 m.

Mean group-sizes (from 25× binocular sightings) of *T. truncatus* for east (9.8, 0.25) and west (10.0, 0.18) regions were similar (Table 2), and had an overall range of 1–68 animals. The mean group size of *S. frontalis* was larger in the east (24.3, 0.19) than the west (15.6, 0.21) with an overall range of 1–267 animals. Group-sizes of *S. bredanensis* were 8, 11, and 20 animals. The east mean group-size for both *T. truncatus* and *S. frontalis* is the size-biased or expected mean group-size because the expected mean was significantly smaller that the arithmetic mean, 10.9 (P=0.14) and 31.9 (P=0.08), respectively.

The most abundant species (number of individuals; CV) found in U.S. GOM OCS waters was *S. frontalis* (30,772; 0.27); the vast majority (91%) occurring in the east (27,997; 0.29). The density of *S. frontalis* was about eight times greater in the east compared to the west (20.1 and 2.6 dolphins/100 km^2, respectively). The abundance of *T. truncatus* was 25,320 (0.26); there was greater abundance in the east (15,198; 0.34) than in the west (10,122; 0.29) but with similar densities (10.9 and 9.5 dolphins/100 km^2, respectively). The total OCS abundance of *S. bredanensis* was 1238 (0.65), and that of *T. truncatus+S. frontalis*, 1868 (0.37).

Discussion

Both *T. truncatus* and *S. frontalis* occur in northern GOM waters outside the OCS (i.e. waters <20 m or >200 m). About 23,000 *T. truncatus* inhabit inshore and coastal waters (≤20 m) (Waring et al., 2001) and nearly 3000 occur in oceanic waters (Mullin and Fulling[2]). Both the "coastal" and "offshore" ecotypes of *T. truncatus* (Hersh and Duffield, 1990) occur in the northern GOM (LeDuc and Curry, 1998). How these ecotypes are distributed in the northern GOM and western North Atlantic is being investigated from skin biopsy samples collected, in part, during the 1998–2001 OCS surveys. Using mitochondrial DNA, obtained from biopsy samples collected during a U.S. Atlantic ship survey, Torres et al. (2003) reported no offshore form was sampled within 6 km of shore and no coastal from was sampled beyond 39 km from shore or in waters >34 m deep. Forty-seven percent (35/75) of the GOM OCS *T. truncatus* groups were in waters >34 m deep.

Ship surveys of northern GOM waters indicate that very few *S. frontalis* (<500 animals) occur in oceanic waters, and those that do are usually found close to the shelf edge in waters <500 m deep (Davis et al., 1998; Mullin and Fulling[2]). The smaller "offshore" or "Gulfstream" *S. frontalis* that occurs in parts of the oceanic Atlantic (Perrin, 2002) has not been recorded for the northern GOM. During the 1998–2001 surveys, *S. frontalis* was sighted in waters <20 m deep. However, because sampling was not perpendicular to bathymetry, abundance estimates were not calculated. This species is not known to occur in U.S. GOM inshore waters (Mullin and Hansen, 1999).

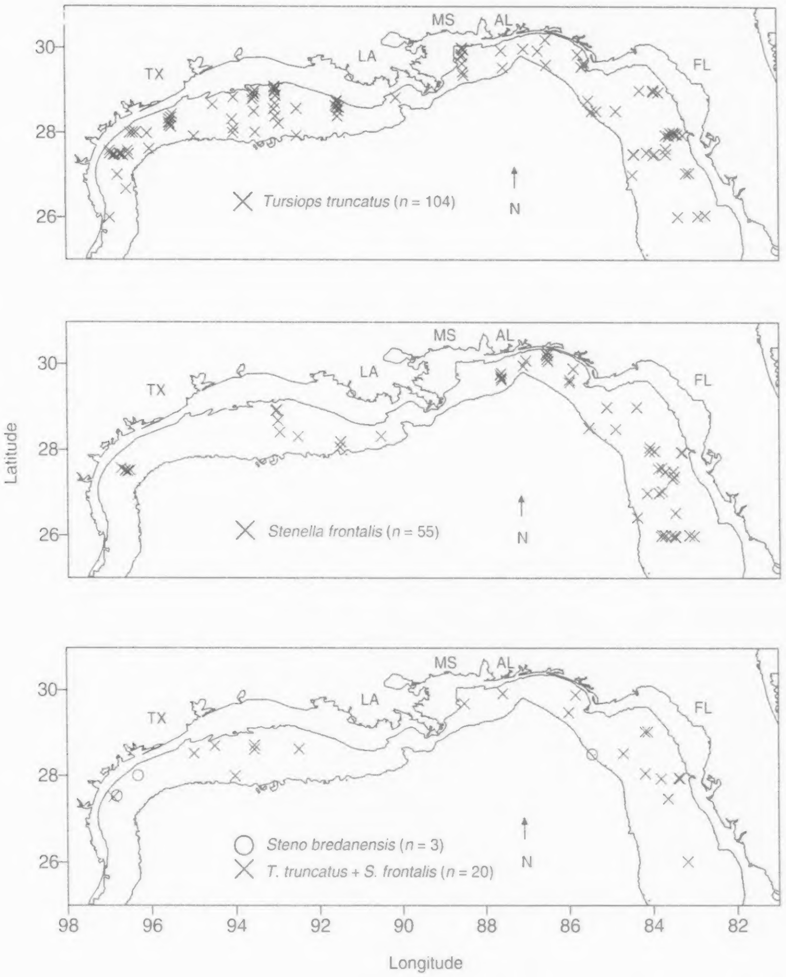

MS
AL
LA
TX
FL
Tursiops truncatus (n = 104)
Stenella frontalis (n = 55)
Steno bredanensis (n = 3)
T. truncatus + S. frontalis (n = 20)
N
30
28
26
Latitude
98
96
94
92
90
88
86
84
82
Longitude

Figure 2

Locations of all on-effort sightings of *Tursiops truncatus* (top), *Stenella frontalis* (center), *Steno bredanensis*, and *T. truncatus+S. frontalis* (bottom) in the northern U.S. Gulf of Mexico outer continental shelf (20–200 m) during fall 1998–2001. Numbers of sightings shown are prior to truncation. The 20- and 200-m isobaths (thin lines) are shown (AL=Alabama, FL=Florida, LA=Louisiana, MS=Mississippi, TX=Texas).

Abundance

The abundance estimates for cetaceans reported in the present study are the first ship-based estimates for the U.S. GOM OCS. Abundance estimates for *T. truncatus* on the OCS (25,320; 0.26) are half the estimate in the pre-2002 SARs (50,247; 0.18) (e.g. Waring et al., 2001), that were based on aerial surveys conducted during fall 1992–94 (Blaylock et al., 1994; Waring et al., 2001). The abundance estimate for *S. frontalis* for the entire U.S. GOM in SARs prior to 2002 (3,213; 0.44) was based on data from ship surveys of OCS and oceanic waters >100 m deep (Waring et al., 2001; Hansen et al.[1]). Our current abundance estimate of *S. frontalis* (30,772; 0.27) for the OCS is almost an order of magnitude larger.

During the 1991–94 aerial surveys, there were 13 sightings of *S. frontalis* groups and 10 sightings that were identified as *T. truncatus+S. frontalis* in OCS waters (SEFSC, NMFS, Pascagoula, MS, unpubl. data). Using these sightings and 139 *T. truncatus* sightings to estimate $f(0)$, we estimated the abundance of *S. frontalis* from the aerial survey data to be 14,866 (0.37) for the U.S. GOM OCS [west, 3,526 (0.86); east, 11,340 (0.40)].

There are several potential reasons for the differences in abundances of the two species from ship and aerial surveys. The U.S. GOM OCS east of 85.5°W makes up about 44% of the U.S. GOM OCS. Aerial survey abundance estimates in this area were based on a small number of transect lines grouped in two places and most of the area was not

Table 2

Group-size, density and abundance estimates of cetaceans in the northern U.S. Gulf of Mexico outer continental shelf (waters 20–200 m deep) during fall 1998–2001 (n=number of group sightings, S=mean group-size, D=animals/100 km^2, N=abundance estimate, CV=coefficient of variation, R=reticle sightings, and K=naked eye sightings).

Species and stratum	n	S	CV(S)	D	N	CV(N)	95% CI
Tursiops truncatus							
East-R	27	9.8	0.25	10.1	14,132	0.40	6426–31,082
East-K	3	6.7	0.70	0.8	1066	0.85	139–8182
East total	30			10.9	15,198	0.38	7442–31,035
West-R	41	10.0	0.18	9.2	9786	0.30	5502–17,406
West-K	4	3.5	0.34	0.3	336	0.67	94–1201
West total	45			9.5	10,122	0.29	5790–17,696
OCS total	75			10.3	25,320	0.26	15,457–41,478
Stenella frontalis							
East-R	32	24.3	0.19	19.5	27,226	0.30	15,093–49,113
East-K	2	11.0	0.09	0.6	771	0.55	252–2358
East total	34			20.1	27,997	0.29	15,978–49,057
West-R	11	15.6	0.21	2.6	2712	0.42	1192–6169
West-K	1	4	—	0.6	63	1.17	9–433
West total	12			2.6	2775	0.41	1279–6023
OCS total	46			12.5	30,772	0.27	18,418–51,412
Steno bredanensis							
East-R	0	—	—	—	0	—	—
East-K	1	11	—	0.4	586	0.85	118–2902
East total	1			0.4	586		
West-R	2	14	0.43	0.6	652	0.98	115–3715
West-K	0	—	—	—	0	—	—
West total	2			0.6	652		
OCS total	3			0.5	1238	0.65	384–3990
T. truncatus+S. frontalis							
East-R	8	2.4	0.22	0.7	983	0.57	324–2983
East-K	0	—	—	—	0	—	—
East total	8			0.7	983		
West-R	8	4.8	0.28	0.8	885	0.47	355–2207
West-K	0	—	—	—	0	—	—
West total	8			0.8	885		
OCS Total	16			0.8	1868	0.37	920–3793

surveyed (see Fig.1 in Baumgartner [1997]). Complete coverage would have certainly led to more *S. frontalis* sightings and it is possible the lines that were surveyed were in areas with more *T. truncatus*. Blaylock and Hoggard (1994) estimated from aerial surveys that about 31% of the *T. truncatus* in OCS waters west of Mobile Bay were in rather a small area from the Mississippi River Delta west to about 90.5°W. Our ship survey effort in this area was small and resulted in only one sighting of *T. truncatus* (Fig. 2). Therefore, our ship-based estimates may have underestimated the abundance of *T. truncatus* in the western OCS. Aerial abundances were based on survey lines that extended from 9.3 km past the 18 m (10 fm) curve to 9.3 km past 183 m (100 fm) curve; therefore the area surveyed was somewhat different than our 20–200 m OCS study area for ship surveys. Aerial survey effort in waters >200 m may have resulted in more sightings of *T. truncatus* than *S. frontalis* because the deeper waters are not the common habitat of *S. frontalis* (Mullin and Fulling[2]) and sightings in waters <20 m would have also been biased toward *T. truncatus*.

Stenella frontalis and *T. truncatus* are similar in length and shape. *Stenella frontalis* are born without spots and become progressively more spotted with age, but young animals look very similar to *T. truncatus* (see Herzing, 1997). Therefore, depending on the composition of the group, from a distance *S. frontalis* are not always easily distinguished from *T. truncatus*; therefore it is possible that some groups were misidentified as *T. truncatus* during aerial surveys, leading to bias in the relative abundance of each species.

The annual PBR for the OCS stock of *T. truncatus* was 432 dolphins, and for the U.S. GOM stock of *S. frontalis*,

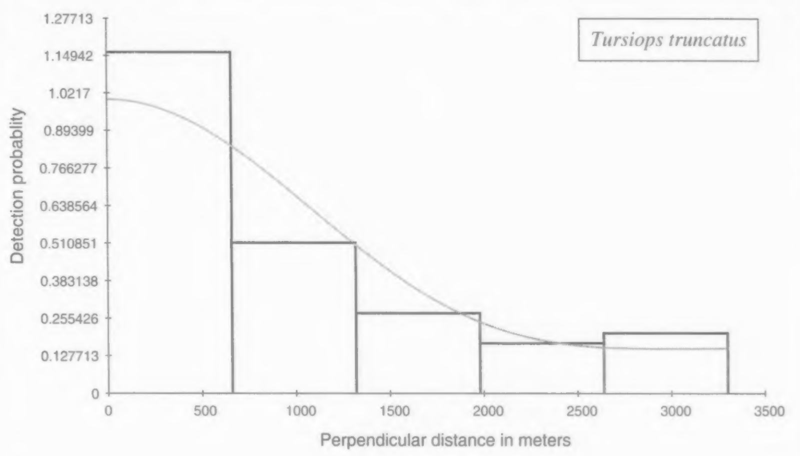


Figure 3

Plot of the detection function of pooled sightings of *Tursiops truncatus* in the northern U.S. Gulf of Mexico.

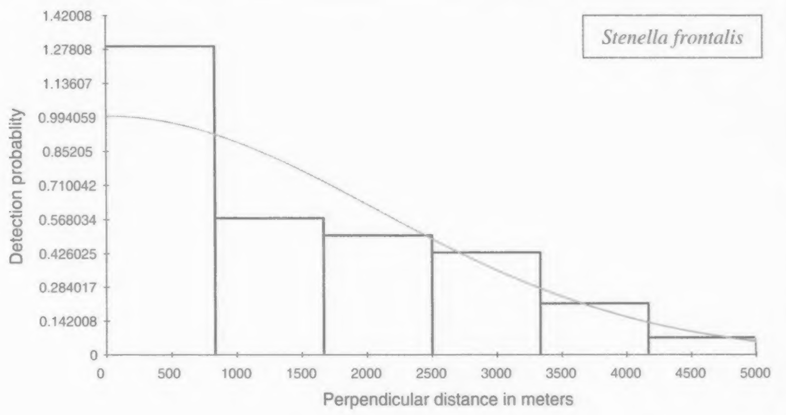


Figure 4

Plot of the detection function of pooled sightings of *Stenella frontalis* in the northern U.S. Gulf of Mexico.

23 dolphins (Waring et al., 2001). Using the abundances, we estimated that the annual PBR would be 204 dolphins for *T. truncatus* and 246 dolphins for *S. frontalis* (Table 2). Although these changes in both PBRs are large, the annual fishery-related mortality and serious injury for each species is estimated to be <3 dolphins in the U.S. GOM OCS (Waring et al., 2001).

Precision

The precision of the abundance estimates for *T. truncatus* (CV=0.26) and *S. frontalis* (CV=0.27) was good, although they were achieved after four years of effort. In cases where there is human-caused mortality in a cetacean stock, abundance estimates with a CV < 0.50 are generally required to avoid incorrectly classifying a cetacean stock as "strategic" under the U.S. MMPA (i.e. annual human-caused mortality>annual PBR) less than 10% of the time (Wade and DeMaster, 1999).

Bias

The surveys were designed to meet the assumptions of line-transect theory (Buckland et al., 2001). However, the abundance estimates were negatively biased because the central assumption that all cetacean groups on the transect line are detected (i.e. $g(0)=1$), certainly was not met, and data were not collected to correct estimates for perception and availability bias (Marsh and Sinclair, 1989). Barlow (1995) estimated perception bias in a ship survey in the Pacific Ocean, and although the group-sizes were not estimated at close range, the majority of groups missed by the primary team were apparently small groups. From this, Barlow (1995) estimated $g(0)$ to range from 0.73 and 0.79 for small groups of delphinids (<21 animals). Delphinids have relatively short dive-cycles but diving synchrony among members of a group can affect availability bias; if dives are asynchronous, the probability that at least one animal will be at the surface increases with group-size. Because availability bias varies by species due to differences in individual dive cycles, group diving behavior, and group-sizes, we were not able to address this potential bias based on Barlow's (1995) results.

The use of the effective strip half-width $[1/f_i(0)]$ from the 25× binocular sightings for the strip width for the strip-transect estimates (Table 2) was assumed to be conservative and somewhat negatively biased. The distance from which animals will come to the ship to ride the bow is unknown and variable, depending on factors such as the animals' previous behavior, number of bowriding opportunities, and the type of ship. If the strip width was too narrow, the strip-transect estimates of abundance would be positively biased.

Our abundance estimates were for the entire U.S. OCS, but the surveys did not extend south of 26.0°N in the eastern Gulf. Sightings from a 1994 survey of the eastern Gulf (Hoffstetter, 2002) indicated that the distribution of *T. truncatus* and *S. frontalis* does not change dramatically between 26.0°N and Key West; therefore we believe this potential bias is minimal.

Because our estimates are from four combined years, another source of bias would occur if there were annual shifts in cetacean distribution, that is, if the majority of animals of any species occurred in a different part of the OCS in one year during fall compared to others years. However, there was no indication that this variation in distribution occurred and therefore potential bias is probably minimal. Potential bias due to the seasonality of the survey is also possible but cannot currently be addressed.

Additionally, survey effort from the 2001 cruise was the most complete effort of all years and may have carried more weight than all the other cruises. However, the 2001 survey provided adequate eastern GOM coverage. Variable survey effort in the fall is common because tropical weather can create rough sea conditions. Additionally, fall surveys always began in the west and terminated in the east. Because the same cruise track was always followed, we rarely had the opportunity to survey those areas not surveyed previously during nighttime transit, and thus may have created both a spatial and temporal bias.

Distribution

The observed distributions of both *T. truncatus* and *S. frontalis* were not surprising given previous descriptions of their distributions. The greater number of *S. frontalis* in the U.S. GOM off Florida compared to the western GOM was suggested by Schmidley and Melcher (1974), and the distribution of sightings reported by Mills and Rademacher (1996) supported this finding. The density of *S. frontalis* was much greater in the eastern GOM OCS than the western GOM OCS but the density of *T. truncatus* was similar in the two regions (Table 2).

The West Florida Shelf and Texas-Louisiana Shelf are very different marine environments, but how habitat differences specifically affect cetacean density patterns is not clear. The oceanography of the U.S. GOM continental shelf is complex, variable both spatially and temporally, and difficult to characterize briefly. Nevertheless, there are some clear distinctions between eastern and western OCS. First, there are 3415 active oil and gas platforms in the U.S. GOM OCS (0–200 m); the vast majority of these platforms (with their attendant boat and helicopter traffic) occur in waters west of Mobile Bay (MMS[5]). Also, ~95% of the U.S. GOM fisheries landings by weight occur west of Mobile Bay (10 years of NMFS[6] data). Additionally, sediment- and nutrient-laden fresh water from the Mississippi River and its distributary, the Atchafalaya River, usually moves west and predominately affects the Texas-Louisiana and Mississippi-Alabama shelves. The bottom of the Texas-Louisiana Shelf is primarily clay-slit mud and sand, and that of the West Florida Shelf is a mosaic of sand, gravel, shell, and coral (Rabalais et al., 1999). Primary production associated with the Mississippi River outflow is the highest measured in the GOM (Lohrenz et al., 1999). However, productivity on the West Florida Shelf can be enhanced by a variety processes (e.g. Gilbes et al., 1996). The deep eastern GOM is subject to the quasi-annual incursion of the Loop Current, which can extend to the Mississippi-Alabama Shelf (Wiseman and Sturges, 1999). This incursion can lead to upwelling episodes along the Loop Current front that may increase productivity along the shelf edge and on the West Florida Shelf (Paluszkiewicz et al., 1983; Gilbes et al., 1996). Baumgartner et al. (2001) reported greater sighting rates of cetaceans in the eastern GOM shelf-edge and oceanic waters and suggested that greater feeding opportunities may occur because of the influence of the Loop Current. Griffin and Griffin (2003), whose study included coastal waters (<20 m), reported that *S. frontalis* on the West Florida Shelf was found in deeper, more saline, and less turbid water than those where *T. truncatus* was found.

Demersal fish (e.g. sciaenids) are abundant and diverse on the western GOM OCS, but less abundant on the eastern OCS (Darnell et al.[7]; Darnell et al.[8]). The known prey of

---

[5] Mineral Management Service, Gulf of Mexico Region website: http://www.gomr.mms.gove/hompg/fastfacts/WaterDepth/WaterDepth.html. [Accessed on 7/8/2003.]

[6] National Marine Fisheries Service web site: http://www.st.nmfs.gov/st1/commercial/. [Accessed on 8 July 2003.]

[7] Darnell, R. M., R. E. Defenbaugh, and D. Moore. 1983. Northwestern Gulf shelf bio-atlas; a study of the distribution of demersal fishes and penaeid shrimp of the soft bottoms of the continental shelf from the Rio Grande to the Mississippi River Delta. Open File Report No. 82-04, 438 p. Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA 70123.

T. truncatus from the GOM consist primarily of demersals, at least close to shore, but they also prey on pelagic species (Barros and Odell, 1990). The prey of *S. frontalis* are not well characterized but descriptions include epipelagic and mesopelagic fish and squid, and benthic invertebrates (Perrin, 2002). Richard and Barbeau (1994) observed "spotted dolphins" feeding on flyingfish (Exocoetidae) in waters 28–35 m deep on the West Florida Shelf. This is not uncommon because *S. frontalis* have been routinely observed feeding on flyingfish at night during haulback of longline gear during NMFS fisheries assessment surveys (Grace[9]). Fertl and Würsig (1995) describe *S. frontalis* feeding on a school of small clupeid fish at the surface south of the Florida Panhandle. A *S. frontalis* satellite-tracked for 24 days off Texas stayed in waters 12–63 m deep (mean, 32.6 m) and 58.1% of its dives were <10 m (Davis et al., 1996). These shallow dives observed by Davis et al. may indicate feeding on epipelagic species.

The occurrence of *S. bredanensis* in continental shelf waters of the U.S. GOM is interesting because this species is usually described as inhabiting oceanic waters (e.g. Jefferson, 2002). In the northern GOM, the estimated density of *S. bredanensis* was larger in OCS waters during fall (0.50 dolphins/100 km^2; Table 2) than that estimated for oceanic waters during spring (0.32 dolphins/100 km^2) (Mullin and Fulling[2]). In fact, if there is no OCS-oceanic shift in distribution between spring and fall, there may be similar numbers of *S. bredanensis* in northern GOM shelf waters (1238; 0.65) as in oceanic waters (1231; 0.45). One of the groups sighted in OCS waters was near the shelf-edge (183 m) but the other two sightings were at depths of 31 m and 33 m off Texas (Fig. 2). The use of shelf waters in the U.S. GOM by this species may not be atypical; two sightings of *S. bredanensis* were made on the West Florida Shelf in waters <55 m deep during August 1994 (Hofstetter, 2002). Pitman and Stinchomb (2002) provide evidence that *S. bredanensis* may be specialized predators of dolphinfish (*Coryphaena hippurus*) in the Pacific Ocean. Dolphinfishes have a circumtropical distribution but occur in oceanic and shelf waters in the northern GOM commonly associated with *Sargassum* and other drifting materials (Hoese and Moore, 1998). *Steno bredanensis* in the northern GOM are commonly found near flotsam, as they are in the Pacific—a place where dolphinfish tend to aggregate.

The abundance estimates presented in this study are the first ship-based estimates of *T. tursiops* and *S. frontalis* from Gulf of Mexico OCS waters. Although probably negatively biased, these estimates provide reliable data for the management of these species. Our results suggest that the diverse U.S. GOM environments provide an excellent natural experiment and opportunity to further understand the ecology of these sympatric cetacean species in OCS pelagic waters.

Acknowledgments

Many people made significant contributions to the success of the surveys including the officers and crews of NOAA ships *Gordon Gunter* and *Oregon II* and the field party chiefs. The marine mammal observers were H. Adams, N. Baertlein, C. Brown, J. Brusher, C. Burks, C. Cates, J. Contillo, L. Csuzdi, A. Debose, A. Beier-Engelhaupt, K. Maze-Foley, J. Henne, W. Hoggard, K. Hough, J. Litz, T. Martinez, M. Newcomer, C. Palmer, K. Rademacher, C. Roden, C. Sinclair, S. Stienessen, J. Tobias, CWH, KDM, and GLF. The comments of two anonymous reviewers improved the manuscript significantly. This work was conducted under Marine Mammal Research permit 779-1339 issued to the SEFSC and supported by Interagency Agreement 15958 between the NMFS, SEFSC and the Minerals Management Service, Gulf of Mexico Region.

Literature cited

Barlow, J.
 1995. The abundance of cetaceans in California waters. Part I: Ship surveys in summer and fall of 1991. Fish. Bull. 93: 1–14.

Barlow, J., S. L. Swartz, T. C. Eagle, and P. R. Wade.
 1995. U.S. marine mammal stock assessments: guidelines for preparation, background, and a summary of the 1995 assessments. NOAA Tech. Memo. NMFS-OPR-6:73.

Barros, N. B., and D. K. Odell.
 1990. Food habits of bottlenose dolphins in the southeastern United States. *In* The bottlenose dolphin (S. Leatherwood and R. R. Reeves, eds.), p. 309–328. Academic Press, San Diego, CA.

Baumgartner, M. F.
 1997. The distribution of Risso's dolphin (*Grampus griseus*) with respect to the physiography of the northern Gulf of Mexico. Mar. Mamm. Sci. 13:614–638.

Baumgartner, M. F., K. D. Mullin, L. N. May, and T. D. Leming.
 2001. Cetacean habitats in the northern Gulf of Mexico. Fish. Bull. 99:219–239.

Blaylock, R. A., and W. Hoggard.
 1994. Preliminary estimates of bottlenose dolphin abundance in the southern U.S. Atlantic and Gulf of Mexico continental shelf waters, 10 p. NOAA Tech. Memo. NMFS-SEFSC-356.

Buckland, S. T., D. R. Anderson, K. P. Burnham, J. L. Laake, D. L. Borchers, and L. Thomas.
 2001. Introduction to distance sampling: estimating abundance of biological populations, 432 p. Oxford University Press, New York, NY.

Davis, R. W., G. S. Fargion, N. May, T. D. Leming, M. Baumgartner, W. E. Evans, L. J. Hansen, and K. D. Mullin.
 1998. Physical habitat of cetaceans along the continental slope in the north-central and western Gulf of Mexico. Mar. Mamm. Sci. 14:490–507.

Davis, R. W., G. A. J. Worthy, B. Würsig, S. K. Lynn, and F. I. Townsend.
 1996. Diving behavior and at-sea movements of an Atlantic spotted dolphin in the Gulf of Mexico. Mar. Mamm. Sci. 12:569–581.

---

[8] Darnell, R. M., J. A. Kleypas, and R. E. Defenbaugh. 1987. Eastern Gulf shelf bio-atlas; a study of the distribution of demersal fishes and penaeid shrimp of the soft bottoms of the continental shelf from the Mississippi River Delta to the Florida Keys. OCS Study 86-0041, 548 p. Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA 70123.

[9] Grace, M. A. 2003. Personal commun. NOAA, 3209 Frederic Street, Pascagoula, MS 39567.

Fertl, D., and B. Würsig.
1995. Coordinated feeding by Atlantic spotted dolphins (*Stenella frontalis*) in the Gulf of Mexico. Aquat. Mamm. 21(1):3–5.

Gilbes, F., C. Tomas, J. J. Walsh, and F. E. Müller-Karger.
1996. An episodic chlorophyll plume on the West Florida Shelf. Cont. Shelf Res. 16:1201–1224.

Griffin, R. B., and N. J. Griffin.
2003. Distribution, habitat partitioning and abundance of Atlantic spotted dolphins, bottlenose dolphins, and loggerhead sea turtles on the eastern Gulf of Mexico continental shelf. Gulf Mex. Sci. 21:23–34.

Hersh, S. L., and D. A. Duffield.
1990. Distinction between northwest Atlantic offshore and coastal bottlenose dolphins based on hemoglobin profile and morphometry. *In* The bottlenose dolphin (S. Leatherwood and R. R. Reeves, eds.), p. 129–142. Academic Press, San Diego, CA.

Herzing, D. L.
1997. The life history of free-ranging Atlantic spotted dolphins (*Stenella frontalis*): age classes, color phases and female reproduction. Mar. Mamm. Sci. 13:576–595.

Hoese, H. D., and R. H. Moore.
1998. Fishes of the Gulf of Mexico, Texas, Louisiana, and adjacent waters, 422 p. Texas A&M Univ. Press, College Station, TX.

Hoffstetter, T. C.
2002. Distribution and abundance of marine mammals in the northeastern Gulf of Mexico relative to sea surface temperature and depth. M.Sc. thesis, 74 p. Univ. Southern Mississippi, Hattiesburg, MS.

Jefferson, T. A.
2002. Rough-toothed dolphin, *Steno bredanensis.* *In* Encyclopedia of marine mammals (W. F. Perrin, B. Würsig, and J. G. M. Thewissen, eds.), p. 1055–1059. Academic Press, San Diego, CA.

LeDuc, R. G., and B. E. Curry.
1998. Mitochondrial DNA sequence analysis indicates need for revision of the genus *Tursiops*. Rep. Int. Whal. Comm. 47:393.

Lerczak, J. A., and R. C. Hobbs.
1998. Calculating sighting distances from angular readings during shipboard, aerial, and shore-based marine mammal surveys. Mar. Mamm. Sci. 14:590–599.

Lohrenz, S. E., D. A. Wiesenburg, R. A. Arnone, and X. Chen.
1999. What controls primary production in the Gulf of Mexico? *In* The Gulf of Mexico large marine ecosystem (H. Kumpf, K. Steidinger, and K. Sherman, eds.), p. 151–170. Blackwell Science, Malden, MA.

Marsh, H., and D. F. Sinclair.
1989. Correcting for visibility bias in strip transect aerial surveys of aquatic fauna. J. Wildl. Manag. 53:1017–1024.

Mills, L. R., and K. R. Rademacher.
1996. Atlantic spotted dolphins (*Stenella frontalis*) in the Gulf of Mexico. Gulf Mex. Sci. 14:114–120.

Mullin, K. D., and L. J. Hansen.
1999. Marine mammals of the northern Gulf of Mexico. *In* The Gulf of Mexico large marine ecosystem (H. Kumpf, K. Steidinger, and K. Sherman, eds.), p. 269–277. Blackwell Science, Malden, MA.

Paluszkiewicz, T., L. P. Atkinson, E. S. Posmentier, and C. R. McClain.
1983. Observations of a Loop Current frontal eddy intrusion onto the West Florida Shelf. J. Geophys. Res. 88: 9639–9651.

Pitman, R. L., and C. Stinchcomb.
2002. Rough-toothed dolphins (*Steno bredanensis*) as predators of mahimahi (*Coryphaena hippurus*). Pac. Sci. 56: 447–450.

Perrin, W. F.
2002. *Stenella frontalis.* Mamm. Species 702:1–6.

Rabalais, N. N., R. S. Carney, and E. G. Escobar-Briones.
1999. Overview of continental shelf benthic communities of the Gulf of Mexico. *In* The Gulf of Mexico large marine ecosystem (H. Kumpf, K. Steidinger, and K. Sherman, eds.), p. 171–195. Blackwell Science, Malden, MA.

Richard, K. R., and M. A. Barbeau.
1994. Observations on spotted dolphins feeding nocturnally on flying fish. Mar. Mamm. Sci. 10:473–477.

Schmidley, D. J., and B. A. Melcher.
1974. Annotated checklist and key to the cetaceans of Texas waters. Southwest. Nat.18:453–464.

Torres, L. G., P. E. Rosel, C. D'Agrosa, and A. J. Read.
2003. Improving management of overlapping bottlenose dolphin ecotypes through spatial analysis and genetics. Mar. Mamm. Sci. 19:502–514.

Wade, P. R., and R. P. Angliss.
1997. Guidelines for assessing marine mammal stocks: report of the GAMMS workshop April 3–5, 1996, Seattle, WA, 93 p. NOAA Tech. Memo. NMFS-OPR-12.

Wade, P. R., and D. P. DeMaster.
1999. Determining the optimum interval for abundance surveys. *In* Marine mammal survey and assessment methods (G. W. Garner, S. C. Amstrup, J. L. Laake, B. F. J. Manly, L. L. McDonald, and D. G. Robertson, eds.), p 53–66. A. A. Balkema, Rotterdam, Netherlands.

Waring, G. T., J. M. Quintal, and S. L. Swartz (eds.).
2001. U.S. Atlantic and Gulf of Mexico marine mammal stock assessments—2001, 310 p. NOAA Tech. Memo. NMFS-NE-168.

Wiseman, W. J., Jr., and W. Sturges.
1999. Physical oceanography of the Gulf of Mexico: processes that regulate its biology. *In* The Gulf of Mexico large marine ecosystem (H. Kumpf, K. Steidinger, and K. Sherman (eds.), p 77–92. Blackwell Science, Malden, MA.

Würsig, B., S. K. Lynn, T. A. Jefferson, and K. D. Mullin.
1998. Behavior of cetaceans in the northern Gulf of Mexico relative to survey ships and aircraft. Aquat. Mamm. 24: 41–50.

Abundance of horseshoe crabs (*Limulus polyphemus*) in the Delaware Bay area

David Hata

Jim Berkson

Department of Fisheries and Wildlife Sciences
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061-0321
E-mail address (for J. Berkson, contact author): jberkson@vt.edu

In recent years, increasing commercial landings of horseshoe crabs (*Limulus polyphemus*) along the Atlantic coast of the United States have raised concerns that the present resource is in decline and insufficient to support the needs of its user groups. These concerns have led the Atlantic States Marine Fisheries Commission (ASMFC) to implement a fishery management plan to regulate the harvest (ASMFC[1]). In order to properly manage any species, specific management goals and objectives must be established, and these goals depend on the resource users involved (Quinn and Deriso, 1999). Horseshoe crabs present a distinct resource management challenge because they are important to a diverse set of users (Berkson and Shuster, 1999).

Horseshoe crabs lay their eggs on sandy beaches in spring and summer, and migrating shorebirds rely heavily on the eggs to supply the energy required to complete their migration (Rudloe, 1980; Shuster and Botton, 1985; Castro and Myers, 1993; Botton et al., 1994; Myers, 1996; Thompson, 1998; Tsipoura and Burger, 1999). Biomedical companies catch horseshoe crabs for their blood, from which they produce Limulus Amebocyte Lysate (LAL) (Novitsky, 1984; ASMFC[1]). LAL is used to detect contamination of injectable drugs and implantable devices by Gram-negative bacteria and is the most sensitive means available for detecting endotoxins (Novitsky, 1984). Finally, horseshoe crabs are harvested commercially for bait in the American eel (*Anguilla rostrata*), catfish (*Ictalurus* spp.), and whelk (*Busycon* spp.) fisheries (ASMFC[1]).

The goal of the ASMFC fishery management plan is to ensure a sustainable population level that will support the continued use by these diverse ecological, biomedical, and fishing interests (ASMFC[1]). Proper management of the resource requires information on the status and dynamics of the horseshoe crab population (Berkson and Shuster, 1999). However, the status of the population is poorly understood, and there is currently no reliable information on which to base any management scheme. Available fishery-independent surveys were not designed for horseshoe crabs, and are of little or no value in assessing their status (ASMFC[2]). Towards this end, the states of New Jersey, Delaware, and Maryland in conjunction with the ASMFC and the National Fish and Wildlife Foundation, funded a pilot benthic trawl survey for the fall of 2001. Data collected during this pilot trawl survey were used to estimate the horseshoe crab population size in the Delaware Bay area.

Methods

This study was conducted in the vicinity of Delaware Bay, which is the center of abundance for horseshoe crabs on the Atlantic coast (Shuster, 1982). The study area extended from north of Cape May, New Jersey, to south of Ocean City, Maryland (39°10′N to 38°10′N), and from shore out to 22.2 km (Fig. 1). The area was divided into four strata based on distance from shore and topography, both of which influence crab distribution. Distance from shore was considered important because horseshoe crab abundance decreases with depth (Botton and Ropes, 1987a). Therefore, the area was split into an inshore zone from 0 to 5.6 km (0 to 3 nautical miles [nmi]) from shore and an offshore zone from 5.6 to 22.2 km (3 to 12 nmi) from shore. Topography was also considered important because commercial fishermen stated that crabs are more abundant in troughs (Burke[3]; Eutsler[4]; Munson[5]). For this study, troughs were defined as at least 2.4 m deep, no more than 1.8 km wide, and more than 1.8 km long. These dimensions are common for troughs identified as important by the fishermen. The inshore and offshore zones were both further divided into trough and nontrough areas. The resulting strata were inshore trough, inshore nontrough, offshore trough, and offshore nontrough.

The study area was divided into grids of one-minute latitude by one-minute longitude. A grid was considered inshore if the majority of its area was in water and inshore of the 5.6-km dividing line. A grid was considered offshore if the majority of its area was offshore of the 5.6-km dividing line and inshore of the 22.2-km boundary. A grid was also considered a trough if the long axis of a trough passed through the grid.

---

[1] ASMFC (Atlantic States Marine Fisheries Commission). 1998. Interstate fishery management plan for horseshoe crab. Fishery management report no. 32, 58 p. Atlantic States Marine Fisheries Commission. 1444 Eye Street, NW, Sixth Floor, Washington, DC 20005.

[2] ASMFC. 1999. Horsehoe crab stock assessment report for peer review. Stock assessment report No. 98-01 (supplement), 47 p. Atlantic States Marine Fisheries Commission, 1444 Eye Street, NW, Sixth Floor, Washington, DC 20005.

[3] Burke, C. 2001. Personal commun. 25 Cove Drive, North Cape May, NJ 08204.

[4] Eutsler, J. 2001. Personal commun. 11933 Gray's Corner Road, Berlin, MD 21811.

[5] Munson, R. 2001. Personal commun. Box 358, Newport, NJ 08345.

Manuscript approved for publication 6 March 2003 by Scientific Editor.

Manuscript received 22 July 2003 at NMFS Scientific Publications Office.

Fish Bull. 101:933–938 (2003).

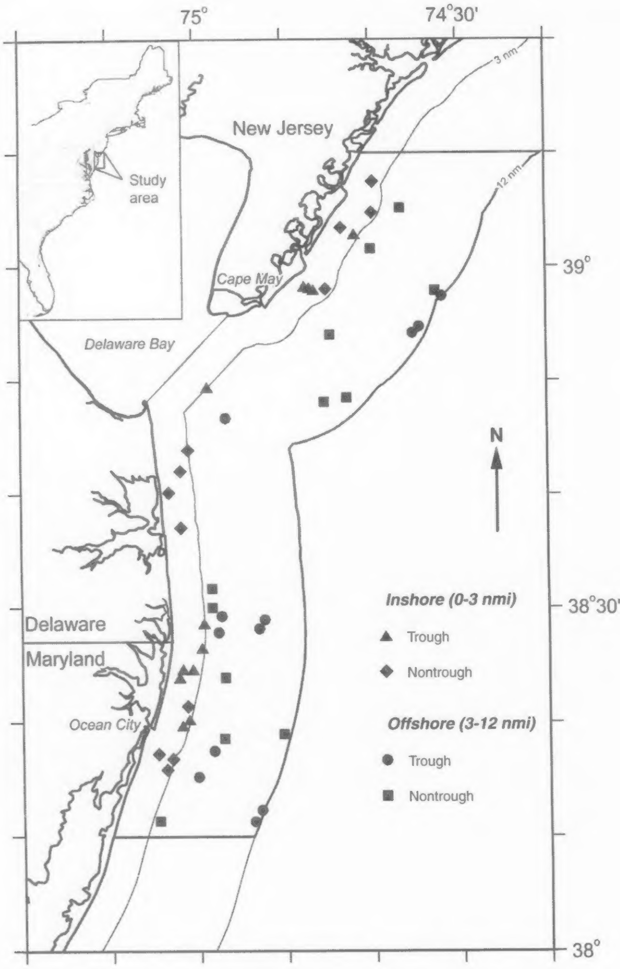


Figure 1

Study area and sampling locations. Symbols indicate type and location of strata. Day and night tows were made at each location.

A grid was considered nontrough if no trough long axis passed through it. Each grid was therefore assigned to one of the four strata. Twelve grids were randomly selected in each stratum, for a total of 48 unique sampling locations. The fishermen also stated that time of day influenced horseshoe crab catchability (Burke[3]; Eutsler[4]; Munson[5]). Therefore, grids were sampled both in daylight and at night. The second tow in a grid (day or night) was made near the location of the first to reduce location variability, but slightly offset to avoid possible influence of the first tow on the catch of the second. The second tow was also made more than 24 hours after the first to avoid interactions, but no more than four days later, to avoid introducing other unknown variability. Abundance estimates from the daytime and nighttime samples were calculated separately for comparison.

Our study was conducted in the fall, between 10 September and 16 October 2001. The stock assessment model adopted by the ASMFC requires abundance information on newly mature crabs, and identification requires that crabs have undergone a terminal molt. Crabs reportedly molt in the late summer and fall in the Delaware Bay area (Burke[3]; Eutsler[4]; Munson[5]).

Sampling was conducted from a chartered 16.8-meter commercial fishing vessel. For capturing horseshoe crabs, commercial fishermen typically use a flounder trawl equipped with a Texas sweep (Burke[3]; Eutsler[4]; Munson[5]; Michels[6]). This modified sweep consists of a chain line instead of rope, which runs from wing to wing of the net (Fig. 2). The net ropeline is attached behind the sweep chain. In addition, usually three rows of weight chain are attached behind the sweep chain. The chain sweep is considered more effective in digging crabs out of the bottom than the typical ground gear of most research trawls. We used a standard two-seam flounder trawl with an 18.3-m headrope and 24.4-m footrope. The net consisted of 14-cm stretched mesh polypropylene throughout and was equipped with chafing gear on the bag. The net was attached to the trawl doors by 91-m ground cables wrapped in rubber cookies. Tow duration was usually 15 minutes (bottom time), except for one tow in the Delaware Bay shipping channel, which was reduced to 7.5 minutes. We assumed that density was not affected by tow duration (e.g. gear saturation was not a factor).

---

[6] Michels, S. 2001. Personal commun. Delaware Department of Natural Resources and Environmental Control, Division of Fish and Wildlife, 89 Kings Hwy., P.O. Box 1401, Dover, DE 19901.

All horseshoe crabs were culled from the catch, and either all or a subsample were examined. For subsamples of a large catch, 50 crabs greater than 150 mm prosomal width were examined, as well as all small, soft, and shedding crabs. Horseshoe crabs that were not examined were counted separately by sex. Examined crabs were measured for prosomal width and identified to sex and maturity. Maturity classifications were as follows: immature; primiparous (mature horseshoe crabs that had not spawned yet); and multiparous (crabs that had spawned at least once [Table 1]). When catches were subsampled, characteristics of examined crabs were extrapolated to all crabs in that tow. Abundance was estimated for each demographic group as well as for the total.

Tow distances were determined for most tows from beginning and ending positions and recorded by using Loran C. These are minima because they do not consider any deviations from a straight path. Distances were not recorded for three tows; therefore they were estimated as the mean distance of all other tows. Net width was estimated as half of the mean of the headrope and footline lengths (Fridman, 1986). The tow distance and net width were used to calculate the swept area to determine the density of horseshoe crabs. We assumed that the ground cables and trawl doors were not effective in catching crabs; therefore all fishing was done only by the net. No information is available on the efficiency of the ground cables or doors for horseshoe crabs, but we do not believe horseshoe crabs are mobile enough, nor swim fast enough, to be effectively herded by them.

The mean density (crabs/km^2) and variance in each stratum were calculated by assuming a Δ-distribution (Aitchison and Brown, 1957; Pennington, 1983), and these estimates were combined by using formulas for a stratified random design (Cochran, 1977). The Δ-distribution model is applicable to skewed data that consist of a portion of zero catches when the frequency of nonzero catches follows a lognormal distribution (Pennington 1983; Pennington 1996). With such skewed data, the estimator of the mean as defined for the Δ-distribution model is more efficient than the sample mean estimator derived from the normal distribution (Smith, 1988). Areas by stratum and total area were substituted for the numbers of grids per stratum and total number of grids for determining stratum weights (Table 2). Latitudinal and longitudinal distances, and therefore grid areas, differed by latitude; therefore grid areas were calculated separately for each minute of latitude. The total number of grids in each stratum was determined for each latitude to calculate the area by stratum and the total area. Ninety-five percent confidence intervals of the stratified mean density and population total were calculated by using the effective degrees of freedom (Cochran, 1977). Mean densities, totals, and confidence limits for demographic groups did not sum to the values calculated by using all horseshoe crabs combined because the stratum mean calculated by the Δ-distribution is a function of the stratum variance, which varies by demographic group.

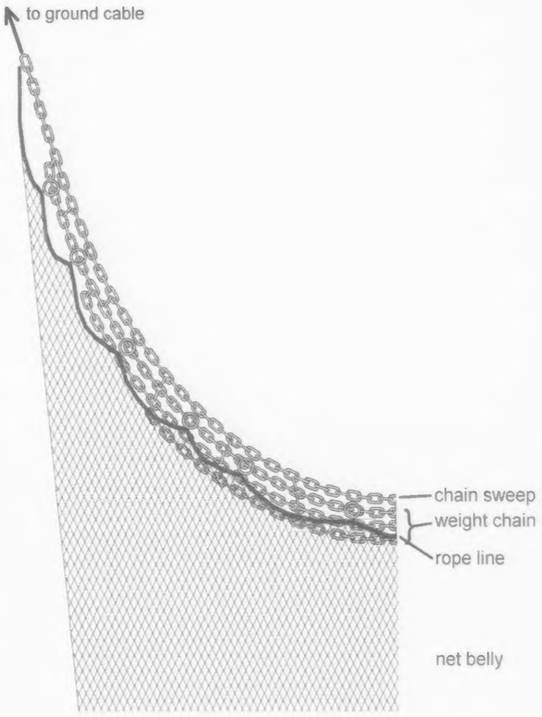


Figure 2

Diagrammatic representation of the Texas sweep used on the flounder trawl.

Results

The mean abundance estimate for all crabs within the study area based on day sampling was 6.81 million crabs within the 2912-km^2 study area (Table 3). The mean abundance estimate for all crabs based on night sampling was 11.40 million crabs in the study area (Table 3).

Table 1

Criteria used in this study for classifying horseshoe crab maturity stage.

	Female	Male
Immature	Gonopores not hard and elevated, no modified pedipalps, soft, membranous area of ventral prosoma (doublure) pale colored.	Hard, elevated gonopores discernible on genital operculum, no modified pedipalps.
Primiparous	Soft, membranous area of ventral prosoma dark colored (indicating presence of eggs), no rub marks on upper opisthosoma.	Gonopores as above, modified pedipalps, both pedipalp digits intact on both sides.
Multiparous	Soft, membranous area of ventral prosoma dark colored, rub marks present on opisthosoma indicating previous amplexus.	Gonopores as above, modified pedipalps, smaller pedipalp digit broken off from at least one side.

Abundance estimates by stage class provided additional information. Multiparous males were estimated at 2.40 million for day sampling and 4.23 million for night sampling. Multiparous females were the next most abundant group, estimated at 1.63 million for day sampling and 2.25 million for night sampling (Table 3).

Primiparous males were uncommon during daylight sampling, estimated at only 84,000 during the day, as compared to 307,000 at night. In contrast, primiparous females were estimated at 338,000 and 361,000 for day and night sampling, respectively.

The estimated abundance of mature males (primiparous and multiparous combined) exceeded that of mature females: 2.48 million to 1.97 million for sampling during the day and 4.54 million to 2.61 million for night sampling. Estimates of immature horseshoe crabs showed that the opposite trend with greater numbers of females than males, 1.34 million to 0.38 million, respectively, for day sampling and 2.31 million to 1.19 million, respectively, for night sampling. With both mature and immature horseshoe crabs, estimates derived from night sampling were higher than those derived from day sampling (Table 3).

Confidence intervals for the estimates were wide, but informative. Confidence limits for total horseshoe crab abundance were 2.29 million to 11.33 million for day sampling and 5.95 million to 16.85 million for night sampling. The lower confidence limits provide useful reference points for conservative, risk-averse management schemes.

Discussion

The study does not estimate actual population size, but rather the total number of horseshoe crabs available to the survey gear. Horseshoe crabs remain at the beaches where they were spawned for the first one to two years of life and gradually disperse offshore as they grow (Rudloe, 1981; Shuster, 1982). Crabs of these early age classes were undoubtedly in shallow shelf waters and coastal embayments beyond the reach of the vessel. Even if they were present, crabs of early age classes may have been too small to be caught in the gear. The study also excluded adults that may have been in shallow waters and embayments. It is also unlikely that 100% of the horseshoe crabs under the gear were in fact captured because some may have been buried too deep in the substrate to have been dug out by the gear. For all of these reasons, abundance estimates can legitimately be considered minimum population estimates. Results can be used as abundance indices for comparison between years, if the study is continued in the future.

Table 2

Horseshoe crab survey stratum sizes. Sampling grids were one minute longitude by one minute latitude. The area of grids sampled in each stratum is denoted by a, the total area (km^2) of the stratum is A, n is the number of grids sampled, and N is the total number of grids in that stratum. Strata are the following: I NT = inshore nontrough, I TR = inshore trough, O NT = offshore nontrough, and O TR = offshore trough.

	Stratum				
	I NT	I TR	O NT	O TR	All
a	32.48	32.51	32.50	32.55	130.04
A	560.07	165.18	1964.87	222.06	2912.17
n	12	12	12	12	48
N	207	61	726	82	1076

The differences between day and night estimates may be the result of horseshoe crabs burying themselves during the day. Alternatively, the horseshoe crabs may be able to detect and avoid the trawl during the day. Night and day collections at individual locations were correlated (r=0.71) suggesting that both were a true reflection of horseshoe crab abundance at that site, although at different levels of efficiency. If the catches were uncorrelated, it would not be possible to determine which, if either, sample accurately represented true abundance. The larger catches and lower

Table 3

Stratified mean density (crabs/km^2), standard deviation (SD), and coefficient of variation of the mean (CV) for horseshoe crab demographic groups and for all crabs combined. Estimated population totals by demographic group and for all crabs combined are given in thousands. UCL and LCL denote upper and lower 95% confidence limits, respectively. Estimates were determined separately for day and night sampling. Because the Δ-distribution was used to calculate stratum means, demographic group values do not sum to those calculated by using all crabs.

	Density (crabs/km^2)			Population total (1000s)		
Demographic group	Mean	SD	CV	Total	UCL	LCL
Day						
Immature females	461	167	0.36	1341	2395	288
Primiparous females	116	40	0.34	338	588	88
Multiparous females	561	126	0.23	1634	2428	839
Immature males	129	45	0.35	377	659	95
Primiparous males	29	7	0.24	84	129	40
Multiparous males	823	207	0.25	2396	3699	1093
All horseshoe crabs	2338	718	0.31	6809	11,326	2291
Night						
Immature females	792	216	0.27	2308	3656	960
Primiparous females	124	26	0.21	361	522	199
Multiparous females	773	145	0.19	2250	3157	1343
Immature males	410	119	0.29	1193	1939	447
Primiparous males	106	40	0.38	307	555	60
Multiparous males	1453	353	0.24	4231	6434	2029
All horseshoe crabs	3915	873	0.22	11,400	16,853	5947

coefficients of variation from the night estimates suggest that the night estimates are more efficient and are probably better estimates of true abundance.

The results of the present study are intermediate between previous estimates of ocean abundance. Botton and Ropes (1987a) estimated that between 2.3 and 4.5 million adults occurred on the continental shelf between New Jersey and Virginia from National Marine Fisheries Service (NMFS) trawl surveys, in contrast to a mean of 7.1 million adults (primiparous and multiparous combined) estimated in the present study area. However, the trawl gear used in the NMFS surveys was inefficient for capturing horseshoe crabs, and the inshore extent was limited by the survey vessel size (Botton and Ropes, 1987a; ASMFC[2]). Botton and Haskin (1984) sampled within 5.6 km of the New Jersey coast using hydraulic clam dredges and obtained horseshoe crab densities of 14,600 to 23,000 per km^2. These densities are much higher than our nighttime estimate of 7900 horseshoe crabs per km^2 (weighted by stratum area) within 5.6 km. The gear we used was probably more efficient in capturing horseshoe crabs than that employed by the NMFS survey but may have been less efficient than the hydraulic dredge. Differing methods between the studies do not allow for a comparison over time.

It is interesting to note that in both the night-based and day-based estimates, females made up the majority of the immature animals, whereas males made up the majority of the mature animals. This could be due to the commercial fishery's preference for harvesting gravid females (Botton and Ropes, 1987b). The continual focused harvest of mature females may reduce their population enough to cause this change in sex ratios. Alternatively, mature females or immature males may have been more abundant outside the study area.

Conclusion

The continuation of annual trawl surveys could allow a full stock assessment to be conducted. The Horseshoe Crab Stock Assessment Subcommittee of the Atlantic States Marine Fisheries Commission has developed a stock assessment plan (HCSAS[7]) based on the catch-survey method derived by Collie and Sissenwine (1983). Unlike age-based stock assessment models, the catch-survey method requires only abundance of primiparous and multiparous horseshoe crabs (HCSAS[7]). The commercial fishery is selective for gravid females (Botton and Ropes, 1987b), and effort is biased toward areas of high abundance (Burke[3]; Eutsler[4]; Munson[5]); therefore commercial data are of limited use for stock assessment. A fishery-independent trawl survey is the best way to provide estimates of abundance while controlling catchability (Hilborn and Walters, 1992; Gunderson, 1993). This study demonstrates the utility of annual trawl surveys to obtain that information.

---

[7] HCSAS (Horseshoe Crab Stock Assessment Subcommittee). 2000. Stock assessment of Atlantic coast horseshoe crabs: a proposed framework, 19 p. A report to the Horseshoe Crab Technical Committee, Atlantic States Marine Fisheries Commission, 1444 Eye Street, NW, Sixth Floor, Washington, DC 20005.

Acknowledgments

This research was funded by the states of New Jersey, Delaware and Maryland through the Atlantic States Marine Fisheries Commission, and by the National Fish and Wildlife Foundation. We are indebted to M. Millard, P. Pooler, D. Smith, and E. Smith for providing statistical advice. We thank J. Brust, P. Himchak, S. Michels, M. Millard, T. O'Connell, and D. Smith of the Horseshoe Crab Stock Assessment and Technical Committees of the ASMFC, and B. Walls and C. N. Shuster Jr. for their input, support, and encouragement in this study. We are grateful to C. Burke, J. Eutsler, and R. Munson for their valuable input regarding horseshoe crab fishing. J. Eutsler and T. Canham provided invaluable assistance in the field. This manuscript was improved by the comments and suggestions of B. Murphy and E. Smith, M. Davis, J. Dew, W. Grogan, L. Hurton, J. McGhee, and A. Williams, and three anonymous reviewers. We greatly appreciate the time and effort of all involved.

Literature cited

Aitchison, J., and J. A. C. Brown.
1957. The log-normal distribution, 176 p. Cambridge Univ. Press, Cambridge, UK.
Berkson, J., and C. N. Shuster Jr.
1999. The horseshoe crab: the battle for a true multiple-use resource. Fisheries 24(11):6–10.
Botton, M. L., and H. H. Haskin.
1984. Distribution and feeding of the horseshoe crab, *Limulus polyphemus*, on the continental shelf off New Jersey. Fish. Bull. 82:383–389.
Botton, M. L., and J. W. Ropes.
1987a. Populations of horseshoe crabs, *Limulus polyphemus*, on the northwestern Atlantic continental shelf. Fish. Bull. 85:805–812.
1987b. The horseshoe crab, *Limulus polyphemus*, fishery and resource in the United States. Mar. Fish. Rev. 49(3): 57–61.
Botton, M. L., R. E. Loveland, and T. R. Jacobsen.
1994. Site selection by migratory shorebirds in Delaware Bay, and its relationship to beach characteristics and abundance of horseshoe crab (*Limulus polyphemus*) eggs. Auk 111:605–616.
Castro, G., and J. P. Myers.
1993. Shorebird predation on eggs of horseshoe crabs during spring stopover on Delaware Bay. Auk 110:927–930.
Cochran, W. G.
1977. Sampling techniques, 3rd ed., 428 p. John Wiley and Sons, Inc., New York, NY.
Collie, J. S., and M. P. Sissenwine.
1983. Estimating population size from relative abundance data measured with error. Can. J. Fish. Aquat. Sci. 40: 1871–1879.
Fridman, A. L.
1986. Calculations for fishing gear designs, 241 p. Fishing News Books, Ltd., Farnham, UK.
Gunderson, D. R.
1993. Surveys of fisheries resources, 248 p. John Wiley and Sons, Inc., New York, NY.
Hilborn, R., and C. J. Walters.
1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty, 570 p. Chapman and Hall, New York, NY.
Myers, J. P.
1996. Sex and gluttony on Delaware Bay. Nat. Hist. 95: 68–77.
Novitsky, T. J.
1984. Discovery to commercialization: the blood of the horseshoe crab. Oceanus 27:13–18.
Pennington, M.
1983. Efficient estimators of abundance, for fish and plankton surveys. Biometrics 39:281–286.
1996. Estimating the mean and variance from highly skewed marine data. Fish. Bull. 94:495–505.
Quinn, T. J., II, and R. B. Deriso.
1999. Quantitative fish dynamics, 542 p. Oxford Univ. Press, New York, NY.
Rudloe, A.
1980. The breeding behavior and patterns of movement of horseshoe crabs, *Limulus polyphemus*, in the vicinity of breeding beaches in Apalachee Bay, Florida. Estuaries 3:177–183.
1981. Aspects of the biology of juvenile horseshoe crabs, *Limulus polyphemus*. Bull. Mar. Sci. 31:125–133.
Shuster, C. N., Jr.
1982. A pictorial review of the natural history and ecology of the horseshoe crab, *Limulus polyphemus*, with reference to other Limulidae. *In* Physiology and biology of horseshoe crabs (J. Bonaventura, C. Bonaventura, and S. Tesh, eds.), p. 1–52. Alan R. Liss, Inc., New York, NY.
Shuster, C. N., Jr., and M. L. Botton.
1985. A contribution to the population biology of horseshoe crabs, *Limulus polyphemus* (L.), in Delaware bay. Estuaries 8:363–372.
Smith, S. J.
1988. Evaluating the efficiency of the Δ-distribution mean estimator. Biometrics 44:485–493.
Thompson, M.
1998. Assessments of the population biology and critical habitat for the horseshoe crab, *Limulus polyphemus*, in the South Atlantic Bight. M.S. thesis, 136 p. Medical Univ. South Carolina, Charleston, SC.
Tsipoura, N., and J. Burger.
1999. Shorebird diet during spring migration stopover on Delaware Bay. Condor 101:635–644.

Use of pop-up satellite archival tags to demonstrate survival of blue marlin (*Makaira nigricans*) released from pelagic longline gear

David W. Kerstetter
Virginia Institute of Marine Science
The College of William & Mary
Rt. 1208 Greate Road
Gloucester Point, Virginia 23062
E-mail address: bailey@vims.edu

Brian E. Luckhurst
Division of Fisheries
Department of Agriculture and Fisheries
P.O. Box CR 52
Crawl CRBX, Bermuda

Eric D. Prince
National Marine Fisheries Service
75 Virginia Beach Drive
Miami, Florida 33146

John E. Graves
Virginia Institute of Marine Science
The College of William & Mary
Rt. 1208 Greate Road
Gloucester Point, Virginia 23062

Blue marlin (*Makaira nigricans*) support commercial and recreational fisheries throughout the tropical and subtropical waters of the Atlantic Ocean. The species is taken in directed recreational and artisanal fisheries in several areas and constitutes an incidental catch of the widespread commercial pelagic longline fishery. Although blue marlin comprise only a small fraction of the catch of the pelagic longline fishery that targets tunas and swordfish, this fishery accounts for the majority of fishing mortality on Atlantic blue marlin (ICCAT, 1997; 2001).

Atlantic blue marlin were last assessed in 2000 by the Standing Committee for Research and Statistics (SCRS) of the International Commission for the Conservation of Atlantic Tunas (ICCAT). The assessment indicated that the total biomass of Atlantic blue marlin is only about 40% of that necessary to produce maximum sustainable yield (MSY) and that current harvests are more than twice the replacement yield, further contributing to the decline of the stock (ICCAT, 2001). A reduction in fishing mortality of approximately 60% is needed simply to halt the decline in stock abundance (Goodyear, 2000).

One means of reducing fishing mortality on blue marlin, without severely impacting catches of target species of the pelagic longline fishery, is to release those blue marlin that are alive at the time longline gear is retrieved (hauled back). Jackson and Farber (1998) reported that 48% of blue marlin caught in the Venezuelan longline fishery are alive at the time of haulback. Data from the U.S. observer program between 1992 and 1996 indicate that 66% of blue marlin were released alive from the domestic longline fishery (Lee and Brown, 1998) and U.S. National Marine Fisheries Service (NMFS) data and mandatory pelagic longline logbook submissions between 1987 and 1991 indicate that 59.8% of blue marlin are released alive from commercial pelagic longline gear (Cramer, 1998).

ICCAT has been encouraging the release of live blue marlin for several years, and in 2000 the Commission mandated that all live blue marlin and white marlin be released from commercial longline and purse seine vessels. However, for such a management measure to significantly reduce fishing mortality, released animals must have a reasonably high postrelease survival rate.

Little information exists about postrelease survival of blue marlin, especially of animals taken on pelagic longline gear. In general, recovery rates of billfish tagged with conventional (streamer) tags have been quite low (<2%; Jones and Prince, 1998; Ortiz et al., 1998). This observation is consistent with high postrelease mortality, although low recovery rates could also result from tag shedding and a lack of reporting recovered tags (Bayley and Prince, 1994; Jones and Prince, 1998). Results of acoustic tracking studies of blue marlin captured on recreational gear suggest that postrelease survival over periods of a few hours to a few days is relatively high, although mortalities have been noted (reviewed in Pepperell and Davis, 1999). More recently, pop-up satellite archival tags (PSATs) have been used to study postrelease survival of blue marlin taken in a recreational fishery. Graves et al. (2002) attached nine PSATs to blue marlin caught on recreational gear off Bermuda. Eight of the tags detached from the animals and reported as expected after five days; net displacement and direction, tag inclination, and temperature data for all eight individuals were consistent with postrelease survival for five days.

In this note, we present the results of a study evaluating the postrelease survival of blue marlin from pelagic longline fishing gear in the western North Atlantic. We include analyses of the movement and behavior of these animals for tagging periods of both five days and thirty days.

Manuscript approved for publication
9 March 2003 by Scientific Editor.
Manuscript received 12 July 2003
at NMFS Scientific Publications Office.
Fish.Bull. 101:939–948 (2003).

Materials and methods

Pop-up satellite tags

There has been rapid development in PSAT technology over the past few years, and there are currently several PSAT models commercially available from different vendors. PSATs vary in many features: in the number of functions (temperature, pressure, tag inclination, light level) they measure; in the parameters and release time for which they can be programed; in the onboard data that they can manipulate and store, in the data they transmit, in their emergency release mechanisms or emergency programming (or both), and in their cost. PSATs have been used on several large pelagic teleosts, including North Atlantic bluefin tuna (e.g. Block et al., 1998; Lutcavage et al., 1999), swordfish (Sedberry and Loefer, 2001), and blue marlin (Graves et al., 2002).

The two tag models used to evaluate postrelease survival of blue marlin in this study—the Microwave Telemetry Inc. (Columbia, MD) PTT-100 (5-day tag) and the Wildlife Computers (Redmond, WA) PAT (30-day tag), are similar in external appearance. Both are slightly buoyant, measure approximately 38 cm by 4 cm diameter (including antenna) and weigh between 65 and 75 g (air weight minus attachment leader and tag head). The size of these tags is sufficiently small as to not appear to impose a major drag on a large marine teleost such as a blue marlin (Block et al., 1998). The greatest exterior differences between the two tag types are their color (the 5-day tags are black and the 30-day tags are grey and white) and the presence of a small metal emergency release mechanism on the attachment leader of the 30-day tag. Both tag models can withstand a minimum pressure equivalent to a depth of about 1000 meters, which is well below the maximum observed depth of previous acoustic tracking analyses of blue marlin movements (Holland et al., 1990; Block et al., 1992).

The 5-day tags (n=7; described in this paper as 5D-1 through 5D-7) were programmed by the manufacturer to detach from the fish 122 hours after activation. A release time of five days was chosen for this PSAT because several blue marlin tagged with conventional tags have been recaptured within five days, demonstrating a return to feeding behavior (E. Prince, unpubl. data). Moreover, mortalities of released blue marlin that have been observed with acoustic telemetry have occurred within 48 hours of release (Pepperell and Davis, 1999). The 5-day tags measured water temperature once an hour and stored the average of two hourly values, for a total of 61 temperature means (from 122 measurements).

The 5-day tags also reported two average inclinometer values, one before the tag released from the fish and one after. These values can be used to infer active forward movement by the fish. This instrument measures the percentage of deployment time that the tag was at an attitude of less than 30° above horizontal by taking a reading every two minutes and either adding or subtracting this percentage from the running total value. Because this value is bracketed with maximum (255) and minimum (0) boundaries, and measurements are taken at short-duration intervals, a mortality or tag shedding event displaying a lack of forward movement will show clearly as an almost vertical reading, even if the fish was initially quite active.

The 30-day tags (n=2; described within this paper as 30D-1 and 30D-2) were programmed by the user to release 32 days following deployment, allowing for a full 30 days of data collection. The release time for the 30-day tags was chosen, in part, to test the assumption that five days was a sufficient duration to capture the rate of postrelease mortality resulting from interaction with longline gear, and to obtain more detailed behavioral data over a longer time interval. It should be noted, however, that a longer release time may bias estimates of postrelease survival because there is an increased period for tag malfunction, physical damage to the tag, or other sources of mortality to occur (Goodyear, 2002). Using the manufacturer's software (PatHost programming software, version 2.06, Wildlife Computers) the 30-day tag was programmed to record temperature values (sensitivity=0.05°C) every minute, and the data were collated for transmission as the fraction of time during each hour-long period that the tag was within each of 12 user-defined bins between the following temperatures (°C): ≤5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, and 60 (for 30D-1) and ≤7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5, and 60 (for 30D-2). Similarly, direct pressure measurements (measured sensitivity=0.5 m) were taken every minute and collated for transmission as the percentage of time during each hour that the tag was within each of 12 user-defined categories between the following depths (m): <0, 2.5, 5, 10, 15, 25, 50, 100, 250, 500, 750, and 1000 (30D-1 and 30D-2). These two tags also recorded minimum and maximum depths and temperatures for each hour during deployment. Finally, the tags recorded light level measurements every minute, and these data were used to calculate a local time of midnight and duration of daylight, thereby allowing a later estimate of daily position.

Analyses

The slightly buoyant tags detached from the fish after the specified intervals, floated to the surface, and transmitted archived data to satellites in the Argos satellite system. Position information and sections of stored data were captured with each satellite pass (Arnold and Dewar, 2001), transmitted to a ground station, and ultimately to the authors by means of the Internet. Location data were analyzed by using the computer program PROGRAM INVERSE (version 2.0, National Geological Survey, 1975; modified by M. Ortiz, NMFS Southeast Fisheries Science Center, Miami, FL) to determine net direction and minimum net displacement from the point at which the blue marlin was tagged and released to the position of the tag at the time of the first precise position (location code 1, 2, or 3) determined by the Argos satellite system. Temperature data from the 5-day tags deployed off Florida were categorized by time of collection as daylight, nighttime, or a composite dawn and dusk period (one hour before and after sunrise and one hour before and after sunset), for analysis of temperature by light level using a one-way ANOVA (Zar, 1999).

Tag deployment

Four commercial longline vessels (48–70 feet LOA [length overall]) in the western North Atlantic Ocean were used for the present study. All carried approximately 20 miles of longline on one large spool, centrally mounted amidships. Gear was set off the stern and retrieved from a hauling station located on one side, approximately amidships—a standard vessel configuration for the U.S. Gulf of Mexico and east coast swordfish fleet. Fishing gear was also typical for vessels in this fleet. A mainline of single-strand monofilament was used and a variety of terminal gear configurations. Different lengths of dropper lines or numbers of hooks between floats (or both) were used in attempts by captains to increase billfish catch rates, although all modifications to the gear were within the range of terminal gear configurations used in this pelagic longline fishery. Leader lengths ranged from 5 to 20 fathoms (9.2–36.6 m), and the buoy drops generally were 10 to 15 fathoms (18.3–27.5 m). Vessels in this study used a combination of "J" hooks (8/0–9/0 sizes) and circle hooks (16/0 size). Bait was either mackerel (Scomber sp.) or squid (*Illex* sp.), and almost every set used chemical lightsticks of varying colors attached to the leader approximately 2 meters above the hook.

PSATs were prepared for attachment to blue marlin prior to departure of the vessels. A large, hydroscopic, surgical-grade nylon tag head was attached to the PSAT with an approximately 20 cm length of 300-pound-test Momoi® brand (Momoi Fishing Co., Ako City, Japan) monofilament line. Metal crimps, covered with black heat-shrink tubing to minimize potential abrasion along the fish's body, were used to attach the monofilament line to the PSAT and the tag head. Individual PSATs were activated and tested at the start of each fishing day prior to the morning haulback. The white flotation bulbs of the 30-day tags were colored black with a permanent marker prior to deployment to decrease their visibility while attached to the fish.

PSATs were attached to all blue marlin that were caught on commercial pelagic longline gear, weighed more than approximately 100 pounds (45.0 kg), and were observed to be in relatively good physical condition, e.g. no large wounds to the viscera. Of the ten blue marlin caught during this study, all but one passed this minimum standard (the one fish that was rejected arrived at the side of the vessel missing the posterior half of its body due to several large bites). Fish were brought alongside the vessels just aft of the hauling station along the rail prior to tagging. Several fish required up to two minutes to become calm enough to be tagged accurately. Many longline captains attempt to save as much leader material as possible from bycatch fishes, and bringing a fish close to the vessel prior to cutting the line is a common practice. The FV *Ark Angel* had a removable section of rail that facilitated tag attachment; on the other three vessels one was forced to lean out over a rail to attach a tag. The average distance between the top of the rail and the fish (freeboard) was approximately one meter. The PSAT tag head was carefully inserted about 5–10 cm below (ventral) the midpoint of the anterior dorsal fin. Tag heads were implanted to a depth of about 8 cm by using a modified tagging applicator approximately 2 meters in length. A conventional streamer tag was attached well posterior of the PSAT by using a standard tagging applicator. Total tagging time, from identification of the fish on the leader as a blue marlin to release following tagging, was less than 5 minutes per fish.

Blue marlin were released by the standard commercial release protocol of cutting the leader near the hook and allowing the hook to remain in the fish. Approximate weights were estimated, and physical location of the hook noted for each blue marlin. An "ACESS" condition scale, analogous to the human neo-natal APGAR test (Apgar, 1953), was developed to quickly evaluate the condition of each fish on a scale of 0–10 by examining five characteristics and assigning each a score of 0–2 (poor to good). These characteristics included overall activity, color, condition of the eyes, stomach eversion, and the general state of the body musculature (presence of bites or lacerations). The time of day, vessel location, and surface water temperature were recorded immediately after tagging. The location of a hooked fish on the longline was also noted in order to estimate the "soak time" of that hook, i.e. the maximum time the fish could have been hooked.

Results and discussion

Seven 5-day PSATs were deployed on blue marlin off Bermuda (n=1) and Florida (n=6) during 2000, and two 30-day PSATs were deployed on blue marlin off North Carolina and Virginia during the summer of 2001 (Tables 1 and 2). Tags were attached onboard four vessels during six trips, ranging from one to eleven fishing days each. The captains all later indicated that the PSAT tagging procedures had minimal interference with normal longlining operations.

Although the captains attempted to target blue marlin by fishing in different areas and with slightly different gear than usual for swordfish trips, the catch rates for this species were not notably different from the catch rates in NMFS data; the catch rate for the trips in 2000 was slightly above average for both season and location, whereas the catch rate in 2001 was below the norm. Of the four trips in 2000, the average CPUE for blue marlin was approximately 0.08 per 100 hooks (7 blue marlin/8650 estimated total hooks), which is comparable to the reported blue marlin CPUE of 0.05 per 100 hooks for the NMFS southeast statistical region for the third quarter of 1998 (Cramer, 2000). For all billfish, excluding swordfish, the CPUE was 0.15 per 100 hooks (12 billfish/8650 estimated total hooks). This value is also comparable to the reported billfish CPUE of 0.12 per 100 hooks for the NMFS southeast statistical region for the third quarter of 1998 (Cramer, 2000). For the three trips in 2001, the blue marlin and billfish CPUEs were lower: 0.04 blue marlin per 100 hooks (3 blue marlin/7780 estimated total hooks) and 0.12 billfish per 100 hooks (9 billfish/7,780 estimated total hooks). These similar catch rates, even when targeting billfish, could be the result of decreased abundance, an inability of the gear to effectively target billfish, or unfavorable water conditions in the study areas.

Of the ten blue marlin caught on the trips, nine were alive and in relatively good physical condition. Based on

Table 1

Summary of pop-up satellite archival tag information for blue marlin (*Makaira nigricans*) released from pelagic longline gear in the western North Atlantic Ocean. Soak time is the maximum time that the blue marlin could have been hooked at that position on the longline. See text for a description of ACESS scores. .

Tag type	Tag number	Soak time (h)	Estimated weight (lb)	ACESS Score	Did tag report?
PTT-100	5D-1	11	200	10	yes
PTT-100	5D-2	12	325	10	yes
PTT-100	5D-3	13	250	10	no
PTT-100	5D-4	14	180	8	yes
PTT-100	5D-5	16	150	9	yes
PTT-100	5D-6	19	275	8	no
PTT-100	5D-7	9	120	10	yes
PAT	30D-1	6	400	10	yes
PAT	30D-2	35	350	9	yes

Table 2

Net movement of blue marlin released from pelagic longline gear in the western North Atlantic Ocean and equipped with pop-up satellite archival tags. Compass direction indicates the bearing from point of release to point of first transmission. Compass direction and straight-line distances between release and first transmission (net movement) were calculated with PROGRAM INVERSE (version 2.0, National Geological Survey, modified by M. Ortiz, NMFS Southeast Fisheries Science Center, Miami, FL). Distances are given in nautical miles (nmi), and kilometers (km). Note that the 30-day tags (30D-1 and 30D-2) did not have inclometers.

Tag number	Location of release	Location of first transmission	Compass direction	Net movement nmi (km)	Net movement per day (km)	Percentage of time less than 30 degrees above horizontal
5D-1	64.99°W, 31.99°N	64.10°W, 30.35°N	155°	107.8 (199.7)	21.2 (39.3)	46.77
5D-2	79.16°W, 28.64°N	79.48°W, 30.81°N	353°	130.8 (242.2)	25.7 (47.6)	47.74
5D-4	79.48°W, 28.59°N	78.37°W, 29.45°N	48°	77.0 (142.6)	15.1 (28.0)	46.77
5D-5	79.41°W, 28.58°N	77.48°W, 28.91°N	79°	103.7 (192.0)	20.4 (37.8)	47.72
5D-7	79.54°W, 28.69°N	78.09°W, 29.77°N	49°	100.0 (185.2)	19.7 (36.4)	47.29
30D-1	74.62°W, 36.79°N	62.10°W, 35.96°N	91°	608.3 (1,126.4)	19.6 (36.3)	—
30D-2	74.61°W, 34.67°N	50.08°W, 40.30°N	67°	1,214.7 (2,249.4)	39.2 (72.6)	—

a 10-point maximum, the ACESS score of the tagged blue marlin ranged from 8 to 10 (Table 1). Of the fish that received a score of less than 10, the primary reason was a loss of color, and minor body musculature lacerations. All blue marlin were hooked in the jaw, whether caught on a "J" hook (n=9) or a circle hook (n=1).

Soak time, the maximum period that the hooked fish could have been on the hook, did not appear to have an effect on either the reporting of the PSAT tags or the ACESS-scored physical condition of the blue marlin (Table 1). The two tags that did not report (5D-3 and 5D-6) were attached to fish with calculated soak times of 13 and 19 hours, respectively, both well under the maximum soak time of 35 hours. The one particularly long soak time (tag 30D-2) resulted from a parting of the mainline during an offshore storm and a subsequent two-day haulback. Boggs (1992) noted that substantial numbers of striped marlin were caught while gear was rising during haulback. Assuming similar behavior among other istiophorid species, this observation may explain the good to excellent condition noted for the blue marlin in our study even after extended soak times. Developing hook-timer technology may allow further description of the relationship, if any, between soak time and the actual length of time that a fish is hooked on the gear.

PSAT performance

The two PSAT models used for our study reflect the rapidly developing technology in this field. The difference in types and amounts of data recorded presented an apparent trade-off between the parameters measured (the resolu-

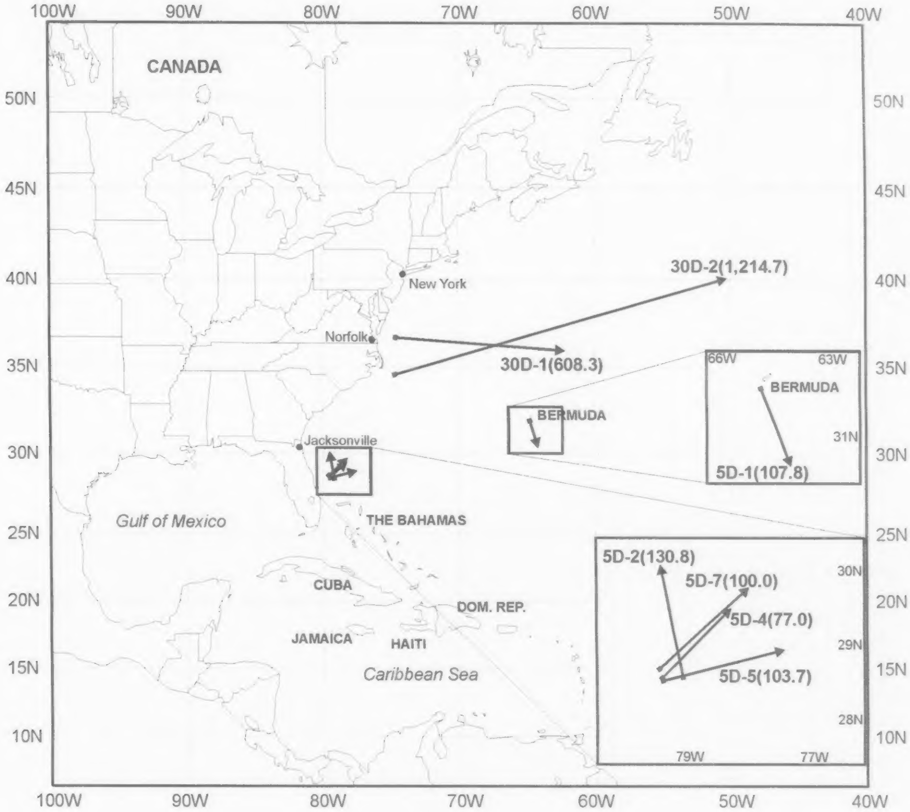


Figure 1

Map showing points of release (squares) and points of pop-up (arrow tips at end of straight lines) for seven of nine blue marlin released from pelagic longline gear in the western North Atlantic Ocean and equipped with pop-up satellite archival tags in 2000 and 2001. The tag identifier for each fish and straight-line distance in nautical miles between point of release and location where tag transmitted data to the Argos satellite are provided in parentheses.

tion of the data), and the probability of recovering (i.e. receiving) all the data recorded, as well as cost. The older, less-expensive 5-day tags stored far fewer data points but transmitted all of them. In contrast, the newer 30-day tags captured far more detailed data, yet only transmitted a fraction of them because of technological constraints, such as a short battery life, file corruption, and occasional problems with satellite uplink. Less than half of the 744 possible hourly histograms were recovered after final processing (46.5% for 30D-1 and 47.6% for 30D-2). However, of the reported histograms that were not corrupted, there was fairly consistent reporting across hours of the day, with an average of 14.75 records (range: 6–23) per hour of day for 30D-1 and 14.42 (range: 8–21) for 30D-2.

The small data storage capacity of the 5-day tag provided information regarding the thermal histories of blue marlin. However, the frequency of temperature recording, and subsequent averaging, limited the utility of the temperature data alone to infer survival. In contrast, the 30-day tags provided higher resolution data on temperature and depth, despite some significant gaps in the time series. In comparing the two tag models used in this study, the less expensive 5-day tags may be sufficient for the specific purpose of evaluating survival, although the greater detail provided by the 30-day tags may be desirable for additional behavioral analyses. Several models of archival satellite tags are currently available—all with differing data collection and storage capabilities. The high costs of tagging-related research, both for equipment and personnel time, therefore require choosing the tag model that is best matched to data needs to answer the specific scientific question of a study.

Net movement

All blue marlin tagged in this study undertook significant movements (Table 2, Fig. 1). The blue marlin released off Bermuda moved away from the islands 199.7 km (107.8 nmi) in a southeast direction over five days. Graves et al. (2002) noted similar movements away from the tagging locations for blue marlin released from the recreational fishery off Bermuda. The four blue marlin tagged off Florida during 2000 moved northeast to east in direction, which is inconsistent with the generally northward flow of the Gulf Stream as it exits the Florida Straits. Both the direction and distance moved by these five fish are evidence of their survival; had these fish been dead and floating, the expected path of movement would have been northward at a velocity of 1.5–3 knots in the Gulf Stream. Of the two fish tagged in 2001, one (30D-1) moved 1126.4 km (608.3 nmi) northeast toward the central north Atlantic, and the other (30D-2) moved 2249.4 km (1214.7 nmi) north toward the Grand Banks.

According to the minimum straight-line distances, blue marlin in this study moved an average of 0.95 nmi/hour (22.9 nmi/day average; range: 15.1–39.2 nmi), which is slightly faster than the average speed of 0.73 nmi/hour (17.6 nmi/day average; range: 10.9–26.4 nmi) reported by Graves et al. (2002). The results of both PSAT studies are consistent with the swimming velocities of 1–2 nmi/hour reported in the acoustic tracking of Pacific blue marlin by Holland et al. (1990), but at the lower end of the range of directly measured swimming speeds of 0.29–4.37 nmi/hour for blue marlin with an acoustic tag reported by Block et al. (1992). However, both measurements from acoustic studies were of short duration deployments. As noted in Block et al. (1992), high initial movement rates would be consistent with actions likely taken by ram-ventilating fish under oxygen debt; slower average speeds over the duration of the deployment could result from the averaging of high initial speed with later slower movements upon the return to normal behavioral patterns.

Forward movement and inclinometer data

The inclinometer values for each of the seven reporting 5-day tags indicated that the PSAT was depressed below 30° above horizontal for an average of 47.25% (range: 46.77–47.74%) of the measurements. These values indicate continual forward movement through the end of the 5-day sampling period and are similar to the observations of Graves et al. (2002), who reported substantial forward movement for more than 40% of their inclinometer measurements. They are also consistent with the observed net displacements of the blue marlin in the present study. Inclinometer values following release for all PSATs indicated that the tags were floating in a vertical position.

Depth and temperature

The temperature data of the 5-day tags indicated numerous vertical movements into cooler water for each fish (Fig. 2, A–E). The maximum temperature recorded by each tag was equivalent to, or slightly greater than, the sea surface temperature (SST) recorded by available SeaWIFS satellite data for that area and date. The slightly higher temperature is possibly an artifact of the 5-day tag; its black coloration may have absorbed heat from direct sunlight while at the surface. Each fish demonstrated movement into slightly cooler waters immediately following release, a behavior also noted in acoustic tracking studies (e.g. Holland et al., 1990; Holts and Bedford, 1993), although it is likely that this movement to cooler waters was still within the upper strata of the water column.

Differences in vertical behavior were noted among individuals. The vast majority (98.6%) of values reported by the 5-day tag on the Bermuda-released fish fell within a range of 2°C (28.6°–30.6°C) (Fig. 2A). This range is smaller than those reported by Graves et al. (2002) for blue marlin in the same general area (range: 26–31°C) but of a much smaller sample size (n=1 vs. n=8 recovered tag datasets). The four blue marlin tagged with 5-day tags off Florida exhibited greater variation in thermal habitat, but their temperature values remained within a range of 6.5°C (Fig. 2, B–E). These four fish also displayed greater vertical movements during the morning hours, especially 5D-3 and 5D-7 (Fig. 2, B and E). For the four blue marlin tagged with 5-day PSATs off Florida in 2000, there was a significant difference between the average temperature during the night, day, and a composite dawn and dusk period (one-way ANOVA; P=0.0003, 2 df).

The 30-day PSATs provided much more detailed temperature information than the 5-day tags, as well as depth data, allowing for a higher resolution of blue marlin vertical movements over the course of a day. The two fish with the 30-day tags spent the vast majority of their total time within the upper five meters of the water column (65.4% for tag 30D-1 and 81.5% for 30D-2, Fig. 3). The higher temperatures recorded by tag 30D-1 are likely due to the warmer surface waters encountered by that fish as it moved toward the central Atlantic, whereas the blue marlin with tag 30D-2 moved northeast into cooler waters near the Grand Banks (Fig. 1). Examination of the temperature histograms generated the by tags for each hour-long period also indicates that these two fish spent all of the first two (30D-1) and six (30D-2) days following release at or near the surface (depth ≤5 m). Only after this initial period did the fish resume the repetitive deep diving behavior seen later during the deployments.

The two 30-day tags deployed in 2001 recorded a broader range of temperatures (30D-1: 29.6–17.8°C and 30D-2: 30.6–16.6°C) than the 5-day tags attached to the blue marlin in 2000. This apparent difference may relate to the measurement protocol of each type of tag. The 5-day tags recorded temperature once an hour and stored the average of two hourly values. In contrast, the 30-day tags recorded temperature values every minute and stored them in the form of an hourly histogram. Inspection of the 30-day tag data revealed that excursions into cooler water were typically of short duration. It is therefore likely that such an excursion could have been missed with a once-an-hour temperature measurement by the 5-day tag. Alternatively, if a lower temperature were encountered at one measurement, it would probably be increased by being averaged with another (likely warmer) measurement from the previous or next hour before being archived. It can be shown mathematically that if the two observations in each pair are independent (which would occur if they are sufficiently separated in time), then the effect of averaging them would be to reduce the variance by one-half. However, cases can be constructed where the observations in each pair would not

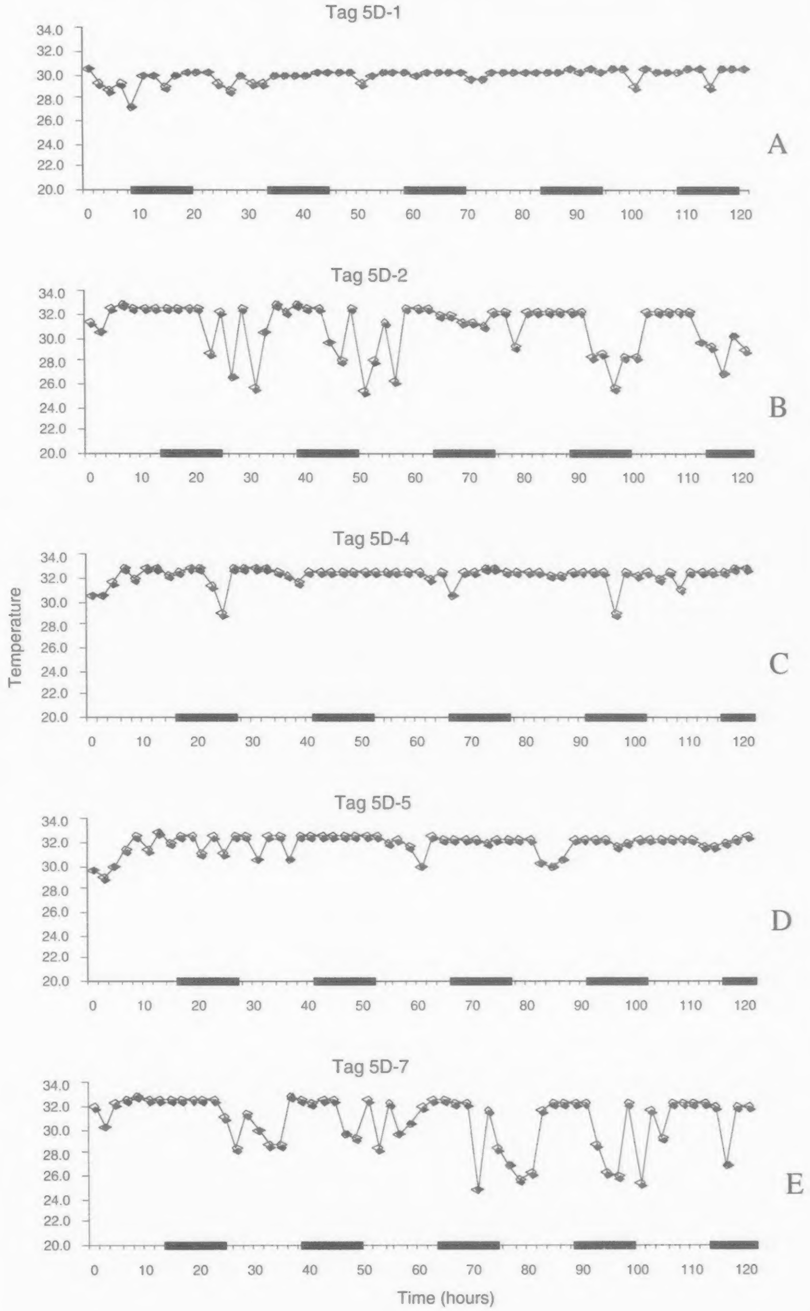


Figure 2

Temperature records for blue marlin released from pelagic longline gear in the western North Atlantic Ocean and equipped with PTT-100 (5-day) pop-up satellite archival tags. Temperature readings were taken once an hour and the average value of two consecutive hours was stored. Temperatures are in degrees centigrade, and the black bars indicate approximate hours of darkness.

be independent. It is therefore not possible to accurately describe the effects (i.e. to reconstruct the actual data or data ranges) of the averaging process of the 5-day tags. Although we feel that any direct comparison of inferred 5-day tag data with the observed data from the 30-day tags would be inappropriate because of the inability to describe this averaging effect, we note that the reported 5-day tag data are not inconsistent with those data from the 30-day tags.

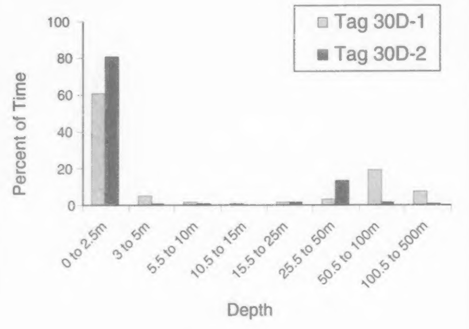


Figure 3

Percentage of total time at depth for blue marlin released from pelagic longline gear in the western North Atlantic Ocean and equipped with PAT (30 day) pop-up satellite archival tags.

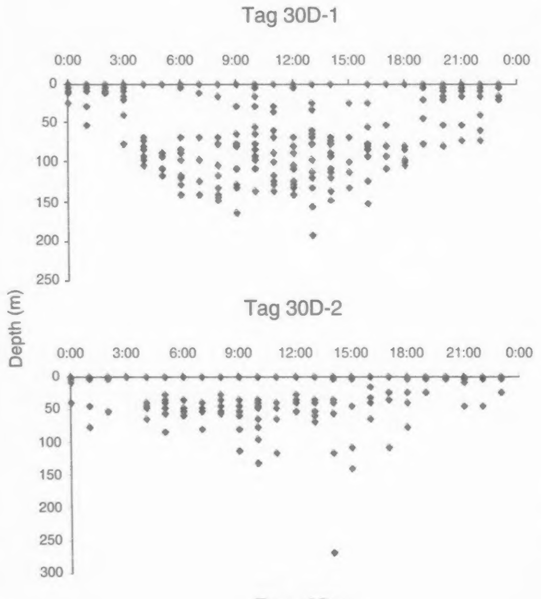


Figure 4

Maximum depths as a function of time of day for blue marlin released from pelagic longline gear in the western North Atlantic Ocean and equipped with PAT (30-day) pop-up satellite archival tags. Both individuals demonstrated a tendency to have greater maximum depths during daylight hours.

The 30-day tags provided considerable information on blue marlin movements at depth. Each hour-long histogram included both the maximum and minimum depths for the hour interval, as well as the percentage of time spent within each predetermined depth bin. For example, during the hour between 10:00 and 11:00 a.m. on 25 July, 30D-1 reported a maximum depth of 28 m and a minimum depth of 0 m, During this hour the fish spent 42 minutes between 0 and 2.5 m, only 72 seconds between 3 and 15 m, and almost 17 minutes between 15.5 and 28 m.

Examination of the maximum and minimum depth values by hour suggests a relationship between movement to depth and daylight hours (Fig. 4). Both blue marlin with 30-day tags made deeper dives during the day than at night. This pattern is similar to diurnal vertical migrations observed in bigeye tuna (*Thunnus obesus*) (Holland et al., 1990; Dagorn et al., 2000), and swordfish (Sedberry and Loefer, 2001) and may relate to feeding within the deep scattering layer.

Billfish survival

Seven of the nine deployed tags successfully reported data. Three independent lines of data transmitted by these tags suggest that seven of the blue marlin carrying these tags and released from pelagic longline gear survived for periods of five or thirty days. First, changes in temperature (5-day tags) or temperature and depth (30-day tags) were consistent with vertical excursions within the water column. Secondly, the direction and magnitude of net displacement cannot be explained by local current patterns and thus imply active movement. Finally, the inclinometer readings (5-day tags only) are consistent with sufficient forward propulsion to depress tags below 30° above horizontal for almost one-half of the measurement periods.

The thermal histories of three fish tagged with 5-day tags (5D-1 off Bermuda and 5D-4 and 5D-5 off Florida) showed little variation (Fig. 2, A, C, and D). Because of the infrequent sampling of temperature by these tags (once an hour, and two hourly values were averaged), it is possible that short-duration dives to cooler waters were not sampled, or that detailed information was lost in the averaging of the two hourly measurements. Alternatively, the thermal histories for these tags could be interpreted as those of fish that died and remained floating at the surface. However, given the preference of individuals for shallow depths recorded by the 30-day tags, even the minimal variations in temperature observed by the 5-day tags may be consistent with movement to depths and postrelease survival. Additionally, the net displacements cannot be attributed solely to local currents (Fig. 1), and inclinometer values indicate continued forward movement throughout the five-day period. We maintain, therefore, that these fish were alive for the whole five-day post-release period.

The reason that two tags did not report data could have been result of mortality (the blue marlin died and sank to a depth at which the tag was crushed or lost positive buoyancy) or of other factors, such as tag malfunction or mechanical damage to the tag. Neither tag contained prerelease software or an emergency release device. There did not appear to be a relationship between the weight of the fish and a nonreporting tag. Both fish with nonreporting tags were hooked in the jaw with "J" hooks. The blue marlin with tag 5D-3 was in excellent condition and actively swam away from the vessel, whereas the fish that received tag 5D-6 was in good condition and actively swam away from the vessel, albeit more slowly than several of the others. It is worth noting that this fish also had an orange streamer tag attached to it from a previous capture, but it was not possible to retrieve this tag without compromising the release protocol.

The results of the present study can be used to generate rough estimates of postrelease survival rate for blue marlin released from the western North Atlantic pelagic longline fishery. This project observed that seven of nine deployed PSATs reported data as programmed (if the two nonreporting tags were indeed mortalities); this results in a 77.8% postrelease survival rate. Combining postrelease mortalities with estimates of mortality at the time of haulback, the compounded mortality for blue marlin conservatively ranges from 65.4% (Jackson and Farber, 1998) to 53.5% (Cramer, 1998). However, the relatively small number of tags deployed in this study clearly limits the general applicability of the these results, as did the prior limitation that only live fish greater than 100 pounds, without clearly mortal wounds, would be tagged. Fortunately, all of the fish encountered alive (one was dead on haulback) met these two requirements. The 77.8% estimate of postrelease survival is nevertheless comparable with the 89% survival rate (8 of 9 reporting PSAT tags) reported by Graves et al. (2002) for blue marlin caught in the recreational fishery off Bermuda—a fishery in which many blue marlin are resuscitated prior to release.

The sample size required for an Atlantic-wide estimate of postrelease mortality would be large given the need to account for different gear configurations, as well as variables such as environmental conditions, season, and geographic area (Goodyear, 2002). Such a project would be extremely expensive with current technology. However, evolving PSAT technology presents the opportunity for additional work describing the interactions between pelagic longline gear and catch species, such as the billfishes. New high-resolution PSATs provide fine-scale data on habitat preferences and behavior, allowing the eventual refinement of models that standardize historical catch-per-unit-of-effort data for pelagic longline gear (e.g. Hinton and Nakano, 1996; Hinton, 2001). Such standardization efforts, if correctly applied, may permit more accurate blue marlin stock assessments (Venizelos et al., 2001).

This study demonstrates that PSAT technology is well suited to estimate postrelease survival rates for blue marlin from the pelagic longline fishery, with minimal interference to normal commercial fishing operations. The relatively high rate of survival of blue marlin inferred from these data for five and thirty days following release from pelagic longline gear demonstrates that management measures requiring live release, such as those recently adopted by ICCAT, can significantly reduce fishing mortality on Atlantic blue marlin.

Acknowledgments

The authors would like to thank Captains Bobby Lamb (FV *Ark Angel*), Keith Garner (FV *Deliverance*), David Kolesar (FV *Triple Threat*), Greg O'Neil (FV *Carol Ann*), and Baker Dunn (FV *Triple Threat*), as well as their crews, for providing assistance in deploying the pop-up tags. We would also like to thank vessel owners Willie Etheridge and Vince Pyle for their assistance in coordinating available vessels. Paul Howey, Roger Hill, and Melinda Braun provided technical assistance with the tags, and Heidi Dewar kindly assisted with the interpretation of the tag data. John Hoenig helpfully assisted with some of the statistical methods. Support for this project was provided by the Bermuda Department of Agriculture and Fisheries, the U.S. National Marine Fisheries Service, and the Hewit Family Foundation.

Literature cited

Apgar, V.
1953. A proposal for a new method of evaluation of the newborn infant. Curr. Res. Anesth. Analg. July–August: 260–265.

Arnold, G., and H. Dewar.
2001. Electronic tags in marine fisheries research: a 30-year perspective. *In* Electronic tagging and tracking in marine fisheries (J. R. Sibert and J. L. Nielsen, eds.), p. 7–34. Kluwer Academic Publishers, Dordrecht, Netherlands.

Bayley, R. E., and E. D. Prince.
1994. A review of tag release and recapture files for *Istiophoridae* from the Southeast Fisheries Science Center's Cooperative Gamefish Tagging Program, 1954 to present. ICCAT (Int. Comm. Conserv. Atl. Tunas) Coll. Vol. Sci. Pap. 41:527–548.

Block, B. A., D. T. Booth, and F. G. Carey.
1998. Depth and temperature of the blue marlin, *Makaira nigricans*, observed by acoustic telemetry. Mar. Biol. 114: 175–183.

Block, B. A., H. Dewar, C. Farwell, and E. D. Prince.
1998. A new satellite technology for tracking the movements of Atlantic bluefin tuna. Proc. Natl. Acad. Sci. U. S. A. 95: 9384–9389.

Boggs, C. H.
1992. Depth, capture time, and hooked longevity of longline-caught pelagic fish: timing bites of fish with chips. Fish. Bull. 90:642–658.

Cramer, J.
1998. Pelagic longline billfish by-catch. ICCAT (Int. Comm. Conserv. Atl. Tunas) Coll. Vol. Sci. Pap. 47:143–154.
2000. Species reported caught in the U.S. commercial pelagic longline and gillnet fisheries from 1996 to 1998. NMFS Sustainable Fisheries Division SFD-99/00-78:1–33.

Dagorn, L., P. Bach, and E. Josse.
2000. Movement patterns of large bigeye tuna (*Thunnus*

obesus) in the open ocean, determined using ultrasonic telemetry. Mar. Biol. (Berlin) 136(2):361–371.

Goodyear, C. P.

2000. Biomass projections for Atlantic blue marlin: potential benefits of fishing mortality reductions. ICCAT (Int. Comm. Conserv. Atl. Tunas) Coll. Vol. Sci. Pap. 52: 1502–1506.

2002. Factors affecting robust estimates of the catch and release mortality using pop-up tag technology. *In* Symposium on catch and release in marine recreational fisheries (A. Studholme, E. D. Prince, and J. Lucy, eds.), p. 172–179. Spec. Pub. Am. Fish. Soc.

Graves, J. E., B. E. Luckhurst, and E. D. Prince.

2002. An evaluation of pop-up satellite tags for estimating post-release survival of blue marlin (*Makaira nigricans*). Fish. Bull. 100:134–142.

Hinton, M.G.

2001. Status of the blue marlin in the Pacific Ocean. Status of the tuna and billfish stocks in 1999. IATTC Stock Assess. Rep. 1:284–318.

Hinton, M.G., and H. Nakano.

1996. Standardizing catch and effort statistics using physiological, ecological, or behavioral constraints and environmental data, with an application to blue marlin (*Makaira nigricans*) catch and effort data from the Japanese longline fisheries in the Pacific. Inter.-Am. Trop. Tuna Comm. Bull. 21(4):171–200.

Holland, K., R. Brill, and R. K. C. Chang.

1990. Horizontal and vertical movements of Pacific blue marlin captured and released using sportfishing gear. Fish. Bull. 88:397–402.

Holts, D. B., and D. W. Bedford.

1993. Horizontal and vertical movements of the shortfin mako shark, *Isurus oxyrinchus*, in the southern California bight. Aust. J. Mar. Freshw. Res. 44(6):901–909.

ICCAT (International Commission for the Conservation of Atlantic Tunas).

1997. Report for the biennial period, 1996–97. Part I (1996), vol. 2, 204 p. ICCAT, Madrid, Spain.

2001. Report of the fourth ICCAT billfish workshop. ICCAT Coll. Vol. Sci. Pap. 53:1–22.

Jackson, T. L., and M. I. Farber.

1998. Summary of at-sea sampling of the western Atlantic Ocean, 1987–1995, by industrial longline vessels fishing out of the port of Cumaná, Venezuela: ICCAT enhanced research Program for billfish 1987–1995. ICCAT Coll. Vol. Sci. Pap. 47:203–228.

Jones, C. D., and E. D. Prince.

1998. The cooperative tagging center mark-recapture database for Istiophoridae (1954–1995) with an analysis of the west Atlantic ICCAT billfish tagging program. ICCAT Coll. Vol. Sci. Pap. 47:311–322.

Lee, D. W., and C. J. Brown.

1998. SEFSC pelagic observer program data summary for 1992–1996. NOAA Tech. Memo. NMFS-SEFSC-408:1–21.

Lutcavage, M. E., R. W. Brill, G. B. Skomal, B. C. Chase, and P. W. Howey.

1999. Results of pop-up satellite tagging of spawning size class fish in the Gulf of Maine: do North Atlantic bluefin tuna spawn in the mid-Atlantic? Can. J. Fish. Aquat. Sci. 56(2):173–177.

Ortiz, M., D. S. Rosenthal, A. Venizelos, M. I. Farber, and E. D. Prince.

1998. Cooperative Tagging Center annual newsletter: 1998. NOAA Tech. Memo. NMFS-SEFSC-423:1–23.

Pepperell, J. G., and T. L. O. Davis.

1999. Post-release behavior of black marlin *Makaira indica* caught and released using sportfishing gear off the Great Barrier Reef (Australia). Mar. Biol. 135:369–380.

Sedberry, G. R., and J. K. Loefer.

2001. Satellite telemetry tracking of swordfish, *Xiphias gladius*, off the eastern United States. Mar. Biol. 139: 355–360.

Venizelos, A., M. I. Farber, and D. D. Benetti.

2001. An evaluation of assumptions associated with blue marlin depth distribution towards the possible incorporation to the standardization of catch and effort statistics for use in stock assessment. ICCAT Coll. Vol. Sci. Pap. 53: 258–262.

Zar, J. H.

1999. Biostatistical analysis, 4th ed., 663 p. Prentice-Hall, Inc., Upper Saddle River, NJ.

Acknowledgment of reviewers

The editorial staff of *Fishery Bulletin* would like to thank the following referees for their time and efforts in providing reviews of the manuscripts published in 2002–2003. Their contributions have helped ensure the publication of quality science.

Dr. Kenneth W. Able
Dr. Peter B. Adams
Dr. Dennis M. Allen
Dr. Allen H. Andrews
Dr. Sharon A. Appleyard
Dr. Freddy Arocha
Dr. Francisco Arreguin-Sanchez
Dr. David W. Au

Mr. John Babaluk
Dr. M. Banks
Dr. Luiz R. Barbieri
Dr. William H. Bayliff
Dr. Terry D. Beachman
Dr. Daniel W. Beckman
Dr. Steven J.M. Blaber
Dr. H.L. Blankenship
Dr. Barbara Block
Dr. James A. Bohnsack
Dr. Jesper Boje
Dr. Steven Branstetter
Dr. Craig Brown
Dr. Barry D. Bruce
Dr. Lewis H. Bullock
Mr. Michael Burton
Dr. Morgan S. Busby
Dr. Mark J. Butler

Dr. Steven X. Cadrin
Dr. Gregor M. Cailliet
Dr. James Carretta
Dr. Patrice Cayre
Dr. Meng-Hsien Chen
Dr. Milani Y. Chaloupka
Dr. Mary Christman
Dr. Mark R. Collins
Dr. Richard E. Condrey
Dr. David O. Conover
Dr. P.D. Cowley
Dr. Paul Crone
Dr. Larry B. Crowder
Dr. D.P. Cyrus

Dr. John M. Dean
Dr. Heidi Dewar
Dr. James G. Ditty
Dr. W.D. Dupaul

Dr. Anne-Marie Eklund
Dr. John M. Emlen
Dr. Charles E. Epifanio
Dr. James A. Estes
Dr. Bruce T. Estrella

Dr. Beatrice Padovani Ferreira
Dr. Richard C. Ferrero
Dr. Clark T. Fontaine
Dr. Alain Fonteneau
Dr. Michael G. Frisk
Dr. Lee A. Fuiman

Dr. Francisco J. Garcia-Rodriguez
Dr. Tony Gharrett
Dr. Vincent F. Gallucci
Mr. Bert Geary
Dr. James Gelsleichter
Dr. Kenneth J. Goldman
Dr. Charles M. Guthrie

Dr. Malcolm Haddon
Dr. Philip S. Hammond
Dr. John Hampton
Dr. Matthew Hare
Ms. Kristen Hart
Dr. Graeme Hays
Dr. Edward J. Heist
Dr. Aaron C. Henderson
Dr. Tyrrell A. Henwood
Dr. Ray Hilborn
Dr. Peter Himchak
Dr. Michael G. Hinton
Dr. John J. Hoey
Dr. Wayne Hoggard
Dr. Kim N. Holland
Dr. John R. Hunter

Dr. James N. Ianelli
Dr. Ana L. Ibanez Aguirre
Dr. Baban S. Ingole

Dr. Larry D. Jacobson
Dr. G. Janacek

Dr. Desmond M. Kahn
Dr. Michel J. Kaiser
Dr. Steven Kalinowski
Dr. Gregory Todd Kellison
Dr. S. Kelly
Dr. Mariano Koen-Alonso
Dr. Christopher C. Koenig
Dr. Nancy E. Kohler

Dr. Han-Lin Lai
Dr. M.D. Lamare
Dr. T.E. Lankford
Dr. Bruce M. Leaman
Dr. Kenneth M. Leber
Dr. W.C. Leggett
Dr. Christopher M. Legault
Dr. Patrick Lehodey
Dr. Jeffrey M. Leis
Dr. R.J.G. Lester
Dr. Karen E. Limburg
Dr. Jason S. Link
Dr. Nancy C.H. Lo
Ms. Linda Lombardi-Carlson
Dr. Peter L. Lutz
Dr. Joanne Lyczkowski-Shultz

Dr. Pamela M. Mace
Ms. Beverly J. Macewicz
Dr. Gary L. Maillet
Ms. Nancy E. Maloney
Dr. Mark Mangel
Ms. Jan Mason
Dr. Bernard J. McConnell
Dr. John McDonald
Mr. Lawrence W. McEachron
Dr. M.G. Meekan
Dr. Richard L. Merrick
Dr. Julian D. Metcalfe
Dr. David L. Meyer
Dr. David A. Milton
Dr. R. Mohan
Dr. Robert K. Mohn
Dr. H. Geoffrey Moser
Dr. Robert G. Muller
Ms. Kristen Munk
Mr. Michael D. Murphy

Dr. Lisa J. Natanson
Mr. David Nemerson
Dr. Nathaniel Newlands
Dr. Stephen J. Newman
Dr. Scott Nichols
Dr. David L. Nieland

Dr. Marcelo E. Oliva
Dr. Jeff Olsen
Dr. Mauricio Ortiz
Dr. Robert S. Otto

Dr. Debra Palka
Dr. Glenn R. Parsons
Dr. Donald E. Pearson

Dr. Michael Pennington
Dr. Pierre Pepin
Dr. James H. Petersen
Dr. Mark S. Peterson
Dr. Daryl J. Pierce
Dr. Eric N. Powell
Dr. Michael H. Prager
Dr. André E. Punt

Dr. Stephen Ralston
Dr. Renata M.A. Ramos
Dr. Carol Reeb
Dr. Carlos Robles
Dr. Jay R. Rooker
Dr. Steven W. Ross
Dr. Sherylynn Rowe

Dr. Susan E. Safford
Dr. Bernard Sainte-Marie
Dr. R.A. Santos
Dr. Kurt M. Schaefer
Dr. Michael Schirripa
Dr. D.C. Schneider
Dr. Kenneth Severin
Dr. Jean-Marie Sevigny
Dr. Richard Shaw
Dr. Kyle Shertzer
Dr. Colin Simpfendorfer
Dr. G.B. Skomal
Dr. Thomas R. Sminkey
Mr. Joseph W. Smith
Dr. Sean Y. Sol
Dr. David A. Somerton
Dr. Patrick J. Sullivan

Dr. C.S. Tamaru
Dr. Mark Terceiro
Ms. Vicky Thayer
Dr. Stephen C. Turner
Dr. W-N Tzeng

Dr. Wolfgang Vogelbein
Dr. Eric Volk

Dr. You-Gan Wang
Dr. Robert D. Ward
Mr. William Watson
Mr. Grant West
Dr. Bradley M. Wetherbee
Dr. Erik Williams
Dr. Sabine Petra Wintner
Dr. David M. Wyanski

Dr. Yongshun Xiao

Mr. Mark Zimmermann

The index and/or table of contents has been removed and photographed separately within this volume year.

For roll film users, this information for the current volume year is at the beginning of the microfilm. For a prior year volume, this information is at the end of the microfilm.

For microfiche users, the index and/or contents is contained on a separate fiche.

Superintendent of Documents **Publications** Order Form

*5178

☐ **YES,** please send me the following publications:

____ Subscriptions to *Fishery Bulletin*
for $55.00 per year ($68.75 foreign)

The total cost of my order is $ _______ . Prices include regular domestic postage and handling and are subject to change.

(Company or Personal Name) (Please type or print)

(Additional address/attention line)

(Street address)

(City, State, ZIP Code)

(Daytime phone including area code)

(Purchase Order No.)

Charge your order. IT'S EASY!

VISA

MasterCard

Please Choose Method of Payment:

☐ Check Payable to the Superintendent of Documents

☐ GPO Deposit Account ☐☐☐☐☐☐☐–☐

☐ VISA or MasterCard Account

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

☐☐☐☐ (Credit card expiration date)

To fax your orders (202) 512-2250

(Authorizing Signature)

Mail To: Superintendent of Documents
P.O. Box 371954, Pittsburgh, PA 15250-7954

Thank you for your order!

This statement is required by the Act of August 12, 1970, Section 3685, Title 39, U.S. Code, showing ownership, management, and circulation of the *Fishery Bulletin*, publication number 366-370, and was filed on 30 September, 2003. The *Bulletin* is published quarterly (four issues annually) with an annual subscription price of $55.00 (sold by the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402). The complete mailing address of the office of publication is NMFS Scientific Publications Office, NOAA, 7600 Sand Point Way NE, BIN C15700, Seattle, WA 98115. The complete mailing address of the headquarters of the publishing agency is National Marine Fisheries Service, NOAA, Department of Commerce, 1335 East-West Highway, Silver Spring, MD 20910. The name of the publisher is Willis Hobart and the managing editor is Sharyn Matriotti; their mailing address is: NMFS Scientific Publications Office, 7600 Sand Point Way NE, BIN 15700, Seattle, WA 98115. The owner is the U.S. Department of Commerce, 14th St. N.W., Washington, DC 20230; there are no bondholders, mortgages, or other security holders. The purpose, function, and nonprofit status of the organization (agency) and the exempt status for Federal income tax purposes has not changed during the preceding 12 months. The extent and nature of circulation is as follows: total number of copies (A) (average number of copies of each issue during the preceding 12 months) was 1490 and the actual number of copies of the single issue published nearest to the filing dates was 1278. Paid circulation (B) is handled by the U.S. Government Printing Office, Washington, DC 20402, and the total number printed for sales (mail subscriptions and individual sales) was 525 for the average number of copies each issue during the preceding 12 months and 475 the actual number of copies of the single issue published nearest to the filing date (C). Free distribution (D) by mail; samples, complimentary and other free copies (average number of copies each issue during the preceding 12 months) was 845 and the actual number of copies of the single issue published nearest to the filing date was 782. Free distribution outside the mail (E) by carriers or other means was 0 for both average number of copies and actual number of copies. Total free distribution (F) was 0 for both average number of copies and actual number of copies of the single issue published nearest the filing date. The total distribution (G: sum of D and B) (average number of copies each issue during the preceding 12 months) was 1370 and the actual number of copies of the single issue published nearest to the filing date was 1257. There were 95 copies (avg. annual) not distributed (H). The total (I: sum of G and H) is equal to the net press run figures shown in Item A: 1490 and 1278 copies, respectively. I certify that the statements made by me above are correct and complete: (Signed) Willis Hobart, Publisher.

Fishery Bulletin

Guidelines for contributors

Content of papers

Articles

Articles are reports of 10 to 30 pages (double spaced) that describe original research in one or a combination of the following fields of marine science: taxonomy, biology, genetics, mathematics (including modeling), statistics, engineering, economics, and ecology.

Notes

Notes are reports of 5 to 10 pages without an abstract that describe methods and results not supported by a large body of data. Although all contributions are subject to peer review, responsibility for the contents of articles and notes rests upon the authors and not upon the editor or the publisher. It is therefore important that authors consider the contents of their manuscripts carefully. Submission of an article is un-derstood to imply that the article is original and is not being considered for publication elsewhere. Manuscripts must be written in English. Authors whose native language is not English are strongly advised to have their manuscripts checked for fluency by English-speaking colleagues prior to submission.

Preparation of papers

Text

Title page should include authors' full names and mailing addresses (street address required) and the senior author's telephone, fax number, e-mail address, as well as a list of key words to describe the contents of the manuscript. **Abstract** must be less than one typed page (double spaced) and must not contain any citations. It should state the main scope of the research but emphasize the author's conclusions and relevant findings. Because abstracts are circulated by abstracting agencies, it is important that they represent the research clearly and concisely. **General text** must be typed in double-spaced format. A brief introduction should state the broad significance of the paper; the remainder of the paper should be divided into the following sections: Materials and methods, Results, Discussion (or Conclusions), and Acknowledgments. Headings within each section must be short, reflect a logical sequence, and follow the rules of multiple subdivision (i.e. there can be no subdivision without at least two subheadings). The entire text should be intelligible to interdisciplinary readers; therefore, all acronyms and abbreviations should be written out and all lesser-known technical terms should be defined the first time they are mentioned. The scientific names of species must be written out the first time they are mentioned; subsequent mention of scientific names may be abbreviated. Follow *Scientific style and format: CBE manual for authors, editors, and publishers* (6th ed.) for editorial style and the most current issue of the *American Fisheries Society's common and scientific names of fishes from the United States and Canada* for fish nomenclature. Dates should be written as follows: 11 November 1991. Measurements should be expressed in metric units, e.g. metric tons (t). The numeral one (1) should be typed as a one, not as a lower-case el (l).

Footnotes

Use footnotes to add editorial comments regarding claims made in the text and to document unpublished works or works with local circulation. Footnotes should be numbered with Arabic numerals and inserted in 10-point font at the bottom of the first page on which they are cited. Footnotes should be formatted in the same manner as citations. If a manuscript is unpublished, in the process of review, or if the information provided in the footnote has been conveyed verbally, please state this information as "unpubl. data," "manuscript in review," and "personal commun.," respectively. Authors are advised wherever possible to avoid references to nonstandard literature (unpublished literature that is difficult to obtain, such as internal reports, processed reports, administrative reports, ICES council minutes, IWC minutes or working papers, any "research" or "working" documents, laboratory reports, contract reports, and manuscripts in review). If these references are used, please indicate whether they are available from NTIS (National Technical Information Service) or from some other public depository. Footnote format: author (last name, followed by first-name initials); year; title of report or manuscript; type of report and its administrative or serial number; name and address of agency or institution where the report is filed.

Literature cited

The literature cited section comprises works that have been published and those accepted for publication (works in press) in peer-reviewed journals and books. Follow the name and year system for citation format. In the text, write "Smith and Jones (1977) reported" but if the citation takes the form of parenthetical matter, write "(Smith and Jones, 1977)." In the literature cited section, list citations alphabetically by last name of senior author: For example, Alston, 1952; Mannly, 1988; Smith, 1932; Smith, 1947; Stalinsky and Jones, 1985. Abbreviations of journals should conform to the abbreviations given in the *Serial sources for the BIOSIS previews database*. Authors are responsible for the accuracy and completeness of all citations. Literature citation format: author (last name, followed by first-name initials); year; title of report or article; abbreviated title of the journal in which the article was published, volume number, page numbers. For books, please provide publisher, city, and state.

Tables

Tables should not be excessive in size and must be cited in numerical order in the text. Headings in tables should be short but ample enough to allow the table to be intelligible on its own. All unusual symbols must be explained in the table legend. Other incidental comments may be footnoted (use italic arabic numerals for footnote markers). Use asterisks only to indicate probability in statistical data. Place table legends on the same page as the table data. We accept tables saved in most spreadsheet software programs (e.g. Microsoft Excel). Please note the following:

- Use a comma in numbers of five digits or more (e.g. 13,000 but 3000).
- Use zeros before all decimal points for values less than one (e.g. 0.31).

Figures

Figures include line illustrations, computer-generated line graphs, and photographs (or slides). They must be cited in numerical order in the text. Line illustrations are best submitted as original drawings. Computer-generated line graphs should be printed on laser-quality paper. Photographs should be submitted on glossy paper with good contrast. All figures are to be labeled with senior author's name and the number of the figure (e.g. Smith, Fig. 4). Use Helvetica or Arial font to label anatomical parts (line drawings) or variables (graphs) within figures; use Times Roman bold font to label the different sections of a figure (e.g. **A**, **B**, **C**). Figure legends should explain all symbols and abbreviations seen within the figure and should be typed in double-spaced format on a separate page at the end of the manuscript. We advise authors to peruse a recent issue of *Fishery Bulletin* for standard formats. Please note the following:

- Capitalize the first letter of the first word of axis labels.
- Do not use overly large font sizes to label axes or parts within figures.
- Do not use boldface fonts within figures.
- Do not create outline rules around graphs.
- Do not use horizontal lines through graphs.
- Do not use large font sizes to label degrees of longitude and latitude on maps.
- Indicate direction of degrees longitude and latitude on maps (e.g. 170°E).
- Avoid placing labels on a vertical plane (except on y axis).
- Avoid odd (nonstandard) patterns to mark sections of bar graphs and pie charts.

Copyright law

Fishery Bulletin, a U.S. government publication, is not subject to copyright law. If an author wishes to reproduce any part of *Fishery Bulletin* in his or her work, he or she is obliged, however, to acknowledge the source of the extracted literature.

Submission of papers

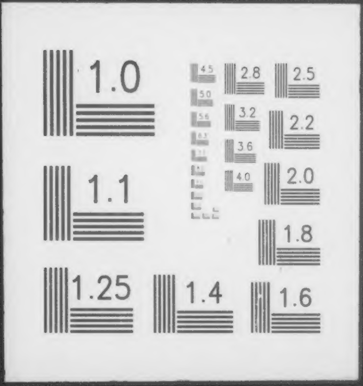
Send four printed copies (one original plus three copies)—clipped, *not stapled*—to the Scientific Editor, at the address shown below. Send photocopies of figures with initial submission of manuscript. Original figures will be requested later when the manuscript has been accepted for publication. Do not send your manuscript on diskette until requested to do so.

Dr. Norman Bartoo
National Marine Fisheries Service, NOAA
8604 La Jolla Shores Drive
La Jolla, CA 92037

Once the manuscript has been accepted for publication, you will be asked to submit a software copy of your manuscript. The software copy should be submitted in WordPerfect or Word format (in Word, save as Rich Text Format). Please note that we do not accept ASCII text files.

Reprints

Copies of published articles and notes are available free of charge to the senior author (50 copies) and to his or her laboratory (50 copies). Additional copies may be purchased in lots of 100 when the author receives page proofs.


1.0
4.5
2.8
2.5
5.0
5.6
3.2
2.2
3.6
4.0
2.0
1.1
1.8
1.25
1.4
1.6